

FORM PTO-1390  
(REV. 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

0020-4850P

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)

09/807470  
NEW

INTERNATIONAL APPLICATION NO.

PCT/JP99/05631

INTERNATIONAL FILING DATE

October 13, 1999

PRIORITY DATE CLAIMED

October 13, 1998

TITLE OF INVENTION

NOVEL PROTEIN WAR-1 AND GENE THEREOF

APPLICANT(S) FOR DO/EO/US

TOHSON, Naoki; YOSHIMA, Tadahiko; KOMIYA, Kazuo; TOJO, Shinichiro; NEMOTO, Kiyomitsu; \*

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). WO 00/22123
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a. ☒ is transmitted herewith.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98-International Search Report (PCT/ISA/210)
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
  - 1.) Fifteen (15) sheets of Formal Drawings
  - 2.) Sequence Listing

\*ISHIKAWA, Hironori; OKUYAMA, Hajime

Form PTO-1390 (REV 11-2000) page 2 of 2

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: TOHDOH, Naoki et al. Conf.:  
Int'l. Appl. No.: PCT/JP99/05631  
Appl. No.: New Group:  
Filed: April 13, 2001 Examiner:  
For: NOVEL PROTEIN WAR-1 AND GENE THEREOF

PRELIMINARY AMENDMENT

**BOX PATENT APPLICATION**

Assistant Commissioner for Patents  
Washington, DC 20231

April 13, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/JP99/05631 which has an International filing date of October 13, 1999, which designated the United States of America.--

09/807470-0360

**IN THE CLAIMS:**

Please amend the claims as follows:

6. (Amended) A protein obtainable by the expression of the DNA of claim 1.

7. (Amended) A recombinant expression vector comprising the DNA of claim 1.

8. (Amended) A recombinant adenovirus vector comprising the DNA of claim 1.

10. (Amended) A DNA which is useful as hybridization probe or PCR primer, which is a single- or double-stranded DNA comprising all <sup>~</sup>of part of the DNA of claim 1, and which makes possible the specific detection of the expression of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3.

12. (Amended) A method for detecting the expression of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, which comprises using the DNA of claim 10 as hybridization probe or PCR primer.

16. (Amended) A pharmaceutical composition comprising the DNA of claim 1 or the protein of claim 6 as an active ingredient.

**IN THE CLAIMS:**

Please amend the claims as follows:

6. (Amended) A protein obtainable by the expression of the DNA of claim 1.

7. (Amended) A recombinant expression vector comprising the DNA of claim 1.

8. (Amended) A recombinant adenovirus vector comprising the DNA of claim 1.

10. (Amended) A DNA which is useful as hybridization probe or PCR primer, which is a single- or double-stranded DNA comprising all or part of the DNA of claim 1, and which makes possible the specific detection of the expression of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3.

12. (Amended) A method for detecting the expression of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, which comprises using the DNA of claim 10 as hybridization probe or PCR primer.

16. (Amended) A pharmaceutical composition comprising the DNA of claim 1 or the protein of claim 6 as an active ingredient.

18. (Amended) A composition for inhibiting proliferation of cancer cells, which comprises the DNA of claim 1 as an active ingredient.

25. (Amended) The composition for facilitating neurotrophic factor secretions of claim 20, wherein the DNA is comprised in a recombinant expression vector.

31. (Amended) A pharmaceutical composition for treating neurodegenerative diseases, which comprises the composition for facilitating neurotrophic factor secretions of claim 20.

**REMARKS**

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

BY  (Reg. No. 21,066)  
for John W. Bailey, #32,881

P.O. Box 747  
Falls Church, VA 22040-0747  
(703) 205-8000

JWB/cqc  
0020-4850P

Attachment: Version With Markings Showing Changes Made

(Rev. 01/22/01)

**VERSION WITH MARKINGS SHOWING CHANGES MADE**

The specification has been amended to provide cross-referencing to the International Application.

The claims have been amended as follows:

6. (Amended) A protein obtainable by the expression of the DNA of [any one of claims 1 to 5]claim 1.

7. (Amended) A recombinant expression vector comprising the DNA of [any one of claims 1 to 5]claim 1.

8. (Amended) A recombinant adenovirus vector comprising the DNA of [any one of claims 1 to 5]claim 1.

10. (Amended) A DNA which is useful as hybridization probe or PCR primer, which is a single- or double-stranded DNA comprising all of part of the DNA of [any one of claims 1 to 5]claim 1, and which makes possible the specific detection of the expression of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3.

12. (Amended) A method for detecting the expression of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, which comprises using the DNA of claim 10 [or 11] as hybridization probe or PCR primer.



16. (Amended) A pharmaceutical composition comprising the DNA of [any one of claims 1 to 5]claim 1 or the protein of claim 6 as an active ingredient.

18. (Amended) A composition for inhibiting proliferation of cancer cells, which comprises the DNA of [any one of claims 1 to 5]claim 1 as an active ingredient.

25. (Amended) The composition for facilitating neurotrophic factor secretions of [any one of claims 20 to 24]claim 20, wherein the DNA is comprised in a recombinant expression vector.

31. (Amended) A pharmaceutical composition for treating neurodegenerative diseases, which comprises the composition for facilitating neurotrophic factor secretions of [any one of claims 20 to 30]claim 20.

13 Rec'd PCT/PTO 11 JUN 2001  
09/807470



BOX SEQUENCE  
PATENT  
0020-4850P

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT(S): TOHDOH, Naoki et al. Conf. No.: 7636  
APPLICATION NO.: 09/807,470 GROUP: 5002  
FILED: April 13, 2001 EXAMINER: W. M. Alvarado  
FOR: NOVEL PROTEIN WAR-1 AND GENE THEREOF

AMENDMENT

Honorable Commissioner of Patents  
Washington, D.C. 20231

June 11, 2001

Sir:

In reply to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures dated May 9, 2001, the following amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE SPECIFICATION:

Please replace the paragraph beginning on page 40, line 18 with the following amended paragraph:

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the homology comparison among the base sequence of

09/807470

cDNAs encoding TRAM from human (HTRAM in the figure)(SEQ ID NO:5), KIAA0057 from human (KIAA0057 in the figure)(SEQ ID NO:12), WAR-1 from human (HWR1 in the figure)(SEQ ID NO:3), and WAR-1 from rat (RWAR1 in the figure)(SEQ ID NO:1).

Please replace the paragraph beginning on page 40, line 23 with the following amended paragraph:

Figure 2 shows the homology comparison among the amino acid sequences deduced from the base sequences of cDNA encoding TRAM from human (HTRAM in the figure)(SEQ ID NO:6), KIAA0057 from human (KIAA0057 in the figure)(SEQ ID NO:13), WAR-1 from human (HWR1 in the figure)(SEQ ID NO:4), and WAR-1 from rat (RWAR1 in the figure)(SEQ ID NO:2).

Please delete the Sequence Listing filed April 13, 2001. Please insert Sequence Listing enclosed herewith immediately after the abstract.

#### REMARKS

Enclosed herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a Substitute Sequence Listing to be inserted into the specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification.

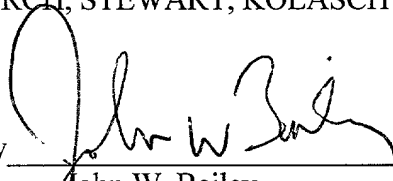
Attached hereto is a marked up version of the changes made to the specification by this amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future submissions, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §1.16 or under 37 C.F.R. §1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By



John W. Bailey  
Reg. No. 32,881

P.O. Box 747  
Falls Church, VA 22040-0747  
(703) 205-8000

JWB/JRK/KW  
0020-4850P

Attachments: Paper and Disk Copy of Substitute Sequence Listing  
Copy of Notice to Comply  
Marked Up Version of Changes Made to The Specification

**VERSION WITH MARKINGS TO SHOW CHANGES MADE TO THE SPECIFICATION**

**BRIEF DESCRIPTION OF DRAWINGS**

Figure 1 shows the homology comparison among the base sequence of cDNAs encoding TRAM from human (HTRAM in the figure)(SEQ ID NO:5), KIAA0057 from human (KIAA0057 in the figure)(SEQ ID NO:12), WAR-1 from human (HWR1 in the figure)(SEQ ID NO:3), and WAR-1 from rat (RWAR1 in the figure)(SEQ ID NO:1).

Figure 2 shows the homology comparison among the amino acid sequences deduced from the base sequences of cDNA encoding TRAM from human (HTRAM in the figure)(SEQ ID NO:6), KIAA0057 from human (KIAA0057 in the figure)(SEQ ID NO:13), WAR-1 from human (HWR1 in the figure)(SEQ ID NO:4), and WAR-1 from rat (RWAR1 in the figure)(SEQ ID NO:2).



04050 0240860



13 Rec'd PCT/PTO 11 JUN 2001  
09/807470

SEQUENCE LISTING

<110> TOHDOH, Naoki et al.

<120> NOVEL PROTEIN WAR-1 AND GENE THEREOF

<130> 0020-4850p

<140> 09/807,470

<141> 2001-04-13

<160> 13

<170> PatentIn version 3.0

<210> 1

<211> 2311

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> (463)..(1554)

<400> 1

agagagagag agagagagag agagagagag agagagagaa atttgatttc cacagcatca 60  
gctccttaag ggaaggtgag attcctaaga gatcagtaga gagcaccagg gagctcgctg 120  
ctgtgttgct atggtgatga tggcaatggt aatgacagtg gcaccagatt tccctgttcc 180  
tggaagccc ctccggtcc cgcggtggg cgcggtggg gatcggtgag gcaaattccg 240  
gctcgacccc gggcctgcgg ggcaggggag cgcggtcgga ttctcttccc tgcctctgca 300  
gccctgtgc gcatgctcgg cctacgcggc cccagccttt gattgatcgg tcggcagcgg 360  
ctgcgaccct gggcggcaga cgggcgggga tggggagccc ggcgctggga gcggcgagct 420  
gatcagcggg ggcggccggg gagtaccggg gagtaccggg gc atg ggg ctc cgc 474  
Met Gly Leu Arg  
1  
aag aag aac gcc agg aac ccc ccg gtg ctg agc cac gaa ttc atg gtg 522  
Lys Lys Asn Ala Arg Asn Pro Pro Val Leu Ser His Glu Phe Met Val  
5 10 15 20  
cag aac cac gcg gat atg gtc tcc tgc gtg ggc atg ttc ttc gtg ctg 570  
Gln Asn His Ala Asp Met Val Ser Cys Val Gly Met Phe Phe Val Leu  
25 30 35  
gga ctt atg ttc gag ggc acg gcc gag atg tcg atc gtg ttc ctc acc 618  
Gly Leu Met Phe Glu Gly Thr Ala Glu Met Ser Ile Val Phe Leu Thr  
40 45 50  
ctg cag cat gga gtc gtt gtc cca gcg gaa ggg cta ccc tcg ggg tcc 666

09807470 "09/807470"



[illegible]

```
<210> 2
<211> 363
<212> PRT
<213> Rattus norvegicus
```



Figure 1 consists of 14 subplots, labeled (a) through (n), each showing the growth of *E. coli* over time (0 to 100 hours) under different conditions. The y-axis for all plots is 'Growth' (0 to 100). The x-axis for all plots is 'Time' (0 to 100). The subplots show that growth is generally high and increases over time, with some variations in the rate and final growth level depending on the condition. The conditions for each subplot are: (a) Control, (b) 10% NaCl, (c) 20% NaCl, (d) 30% NaCl, (e) 40% NaCl, (f) 50% NaCl, (g) 60% NaCl, (h) 70% NaCl, (i) 80% NaCl, (j) 90% NaCl, (k) 100% NaCl, (l) 110% NaCl, (m) 120% NaCl, and (n) 130% NaCl.



gctcagctcg gccctgcct ttgatttatt ttttttctgg gcggccgctg cgaccggga 360  
 ctgacttcgg gatgggaagt ggagcccccg gagctgctac cgtggcggcg gcgctgtgag 420  
 gagcagccag ggggaggcag ctgcggctcg ccggtgagta tccgggaagc gccacc atg 479  
 Met  
 1  
 ggg ctc cgt aag aag agc acc aag aac ccc ccc gtt ctc agc cag gaa 527  
 Gly Leu Arg Lys Lys Ser Thr Lys Asn Pro Pro Val Leu Ser Gln Glu  
 5 10 15  
 ttc atc ctg cag aat cat gcg gac atc gtc tcc tgc gtg ggg atg ttc 575  
 Phe Ile Leu Gln Asn His Ala Asp Ile Val Ser Cys Val Gly Met Phe  
 20 25 30  
 ttc ctg ctg ggg ctt gtg ttc gag gga aca gca gaa gca tcc atc gtg 623  
 Phe Leu Leu Gly Leu Val Phe Glu Gly Thr Ala Glu Ala Ser Ile Val  
 35 40 45  
 ttt ctc act ctt cag cac agt gtt gct gtc cct gca gca gag gaa caa 671  
 Phe Leu Thr Leu Gln His Ser Val Ala Val Pro Ala Ala Glu Glu Gln  
 50 55 60 65  
 gcc acg ggc tca aag tcc ctc tat tat tat ggt gtc aaa gat ttg gcc 719  
 Ala Thr Gly Ser Lys Ser Leu Tyr Tyr Tyr Gly Val Lys Asp Leu Ala  
 70 75 80  
 acg gtt ttc ttc tac atg ctg gtg gca atc att att cat gcc aca att 767  
 Thr Val Phe Phe Tyr Met Leu Val Ala Ile Ile Ile His Ala Thr Ile  
 85 90 95  
 cag gaa tat gtg ttg gat aaa att aac aag aga atg cag ttc acc aaa 815  
 Gln Glu Tyr Val Leu Asp Lys Ile Asn Lys Arg Met Gln Phe Thr Lys  
 100 105 110  
 gcg aaa caa aac aag ttt aac gag tct ggt cag ttt agt gtg ttc tac 863  
 Ala Lys Gln Asn Lys Phe Asn Glu Ser Gly Gln Phe Ser Val Phe Tyr  
 115 120 125  
 ttt ttt tct tgt att tgg ggc aca ttc att tta atc tct gaa aac tgc 911  
 Phe Phe Ser Cys Ile Trp Gly Thr Phe Ile Leu Ile Ser Glu Asn Cys  
 130 135 140 145  
 ctg tca gac cca act ctt ata tgg aag gct cgt ccc cat agc atg atg 959  
 Leu Ser Asp Pro Thr Leu Ile Trp Lys Ala Arg Pro His Ser Met Met  
 150 155 160  
 aca ttt caa atg aag ttt ttc tac ata tcc cag ttg gct tac tgg ttt 1007  
 Thr Phe Gln Met Lys Phe Phe Tyr Ile Ser Gln Leu Ala Tyr Trp Phe  
 165 170 175  
 cat gct ttt cct gaa ctc tac ttc cag aaa acc aaa aaa caa gac atc 1055  
 His Ala Phe Pro Glu Leu Tyr Phe Gln Lys Thr Lys Lys Gln Asp Ile  
 180 185 190

cct cgt caa ctt gtc tac att ggt ctt cac ctc ttc cac att act gga	1103
Pro Arg Gln Leu Val Tyr Ile Gly Leu His Leu Phe His Ile Thr Gly	
195 200 205	
gct tat ctc ttg tac ttg aat cat ttg gga ctt ctt ctt ttg gta ctg	1151
Ala Tyr Leu Leu Tyr Leu Asn His Leu Gly Leu Leu Leu Leu Val Leu	
210 215 220 225	
cat tat ttt gtt gaa tta ctt tcc cac atg tgc ggc ctg ttt tac ttt	1199
His Tyr Phe Val Glu Leu Leu Ser His Met Cys Gly Leu Phe Tyr Phe	
230 235 240	
agt gat gaa aag tac cag aaa ggc ata tct ctg tgg gcc att gtg ttt	1247
Ser Asp Glu Lys Tyr Gln Lys Gly Ile Ser Leu Trp Ala Ile Val Phe	
245 250 255	
atc ttg ggt aga ctt gtg act tta att gtt tcc gta ctc act gtt ggg	1295
Ile Leu Gly Arg Leu Val Thr Leu Ile Val Ser Val Leu Thr Val Gly	
260 265 270	
ttt cac ctg gct gga tcg cag aat cgg aat cct gat gcc ctt act gga	1343
Phe His Leu Ala Gly Ser Gln Asn Arg Asn Pro Asp Ala Leu Thr Gly	
275 280 285	
aat gta aat gtg ttg gca gct aaa att gct gtt ctg tcg tcc agt tgc	1391
Asn Val Asn Val Leu Ala Ala Lys Ile Ala Val Leu Ser Ser Ser Cys	
290 295 300 305	
acg atc caa gcc tac gta aca tgg aac tta att act ctc tgg ctt cag	1439
Thr Ile Gln Ala Tyr Val Thr Trp Asn Leu Ile Thr Leu Trp Leu Gln	
310 315 320	
agg tgg gta gaa gat tct aat att cag gcc tca tgt atg aaa aag aaa	1487
Arg Trp Val Glu Asp Ser Asn Ile Gln Ala Ser Cys Met Lys Lys Lys	
325 330 335	
cgg tcg aga tct tct aaa aaa aga aca gaa aac gga gtg gga gtg gaa	1535
Arg Ser Arg Ser Ser Lys Lys Arg Thr Glu Asn Gly Val Gly Val Glu	
340 345 350	
act tca aat aga gta gac tgt ccg cca aag agg aaa gag aaa tct tca	1583
Thr Ser Asn Arg Val Asp Cys Pro Pro Lys Arg Lys Glu Lys Ser Ser	
355 360 365	
ttaa tctttgcaag cgcattgatt aatgtctgca aaggaatctg ctctttgagg	1636
tttctttctg cactagagat ttttctgttt ttgaaaatag ttctgtctct tcggtttttg	1696
ttattgaact gtttcatgta ttttttaaag acatttgagg ggaggaggat tattatgaat	1756
gggaaaaaaaa gattttgggt gagactaaat tactcatcgt caaaataatg tcaaaatagt	1816
tttggggatc accactatat tttgttttga tttttaacct ttcaacattt toctaagt	1876
ttgcagagat aactgcacaa ttttgcatat caatgatact ggttcttact cccaccagt	1936

tttcataata ctaacaagat ggtctctcct agcaagatta tgtgtttaat gcttgctttg 1996  
 gggtaaaata aaagtacgaa aaagggtggaa gtcaaatacag tattctgtaa ttgttagaat 2056  
 ttattttttta agaacttaca actcagaaaa gattgctaga ctcaccaaaa taataaatgt 2116  
 tctttatttt acaggtagtg attattagtg cttcatcccc atttaaaaaa acacagtact 2176  
 aatgggtaac acatatggag gtttgctgcc atatatttg catcaaaata tcattaatta 2236  
 atataaaaat attaaaatca ttccgtgtcca ttccacttgt aaatgggaat tc 2288

<210> 4  
 <211> 369  
 <212> PRT  
 <213> Homo sapiens

<400> 4

Met Gly Leu Arg Lys Lys Ser Thr Lys Asn Pro Pro Val Leu Ser Gln  
 1 5 10 15

Glu Phe Ile Leu Gln Asn His Ala Asp Ile Val Ser Cys Val Gly Met  
 20 25 30

Phe Phe Leu Leu Gly Leu Val Phe Glu Gly Thr Ala Glu Ala Ser Ile  
 35 40 45

Val Phe Leu Thr Leu Gln His Ser Val Ala Val Pro Ala Ala Glu Glu  
 50 55 60

Gln Ala Thr Gly Ser Lys Ser Leu Tyr Tyr Tyr Gly Val Lys Asp Leu  
 65 70 75 80

Ala Thr Val Phe Phe Tyr Met Leu Val Ala Ile Ile Ile His Ala Thr  
 85 90 95

Ile Gln Glu Tyr Val Leu Asp Lys Ile Asn Lys Arg Met Gln Phe Thr  
 100 105 110

Lys Ala Lys Gln Asn Lys Phe Asn Glu Ser Gly Gln Phe Ser Val Phe  
 115 120 125

Tyr Phe Phe Ser Cys Ile Trp Gly Thr Phe Ile Leu Ile Ser Glu Asn  
 130 135 140



[illegible]

```
<220>
<221> CDS
<222> (122) .. (1246)
```

10

Tyr Ile Ser Asp Pro Thr Ile Leu Trp Arg Ala Tyr Pro His Asn Leu	
145 150 155 160	
atg aca ttt caa atg aag ttt ttc tac ata tca cag ctg gct tac tgg	649
Met Thr Phe Gln Met Lys Phe Phe Tyr Ile Ser Gln Leu Ala Tyr Trp	
165 170 175	
ctt cat gct ttt cct gaa ctc tac ttc cag aaa acc aaa aaa gaa gat	697
Leu His Ala Phe Pro Glu Leu Tyr Phe Gln Lys Thr Lys Lys Glu Asp	
180 185 190	
att cct cgt cag ctt gtc tac att ggt ctt tac ctc ttc cac att gct	745
Ile Pro Arg Gln Leu Val Tyr Ile Gly Leu Tyr Leu Phe His Ile Ala	
195 200 205	
gga gct tac ctt ttg aac ttg aat cat cta gga ctt gtt ctt ctg gtg	793
Gly Ala Tyr Leu Leu Asn Leu Asn His Leu Gly Leu Val Leu Leu Val	
210 215 220	
cta cat tat ttt gtt gaa ttt ctt ttc cac att tcc cgc ctg ttt tat	841
Leu His Tyr Phe Val Glu Phe Leu Phe His Ile Ser Arg Leu Phe Tyr	
225 230 235 240	
ttt agc aat gaa aag tat cag aaa gga ttt tct ctg tgg gca gtt ctt	889
Phe Ser Asn Glu Lys Tyr Gln Lys Gly Phe Ser Leu Trp Ala Val Leu	
245 250 255	
ttt gtt ttg gga aga ctt ctg act tta att ctt tca gta ctg act gtt	937
Phe Val Leu Gly Arg Leu Leu Thr Leu Ile Leu Ser Val Leu Thr Val	
260 265 270	
ggt ttt ggc ctt gca aga gca gaa aat cag aaa ctg gat ttc agt act	985
Gly Phe Gly Leu Ala Arg Ala Glu Asn Gln Lys Leu Asp Phe Ser Thr	
275 280 285	
gga aac ttc aat gtg tta gct gtt aga atc gct gtt ctg gca tcc att	1033
Gly Asn Phe Asn Val Leu Ala Val Arg Ile Ala Val Leu Ala Ser Ile	
290 295 300	
tgc gtt act cag gca ttt atg atg tgg aag ttc att aat ttt cag ctt	1081
Cys Val Thr Gln Ala Phe Met Met Trp Lys Phe Ile Asn Phe Gln Leu	
305 310 315 320	
cga agg tgg agg gaa cat tct gct ttt cag gca cca gct gtg aag aag	1129
Arg Arg Trp Arg Glu His Ser Ala Phe Gln Ala Pro Ala Val Lys Lys	
325 330 335	
aaa cca aca gta act aaa ggc aga tct tct aaa aaa gga aca gaa aat	1177
Lys Pro Thr Val Thr Lys Gly Arg Ser Ser Lys Lys Gly Thr Glu Asn	
340 345 350	
ggt gtg aat gga aca tta act tca aat gta gca gac tct ccc cgg aat	1225
Gly Val Asn Gly Thr Leu Thr Ser Asn Val Ala Asp Ser Pro Arg Asn	
355 360 365	
aaa aaa gag aaa tct tca taa tgaattataa actaattgat t	1267



Lys Lys Glu Lys Ser Ser  
370

<210> 6  
<211> 374  
<212> PRT  
<213> Homo sapiens

<400> 6

Met Ala Ile Arg Lys Lys Ser Thr Lys Ser Pro Pro Val Leu Ser His  
1 5 10 15

Glu Phe Val Leu Gln Asn His Ala Asp Ile Val Ser Cys Val Ala Met  
20 25 30

Val Phe Leu Leu Gly Leu Met Phe Glu Ile Thr Ala Lys Ala Ser Ile  
35 40 45

Ile Phe Val Thr Leu Gln Tyr Asn Val Thr Leu Pro Ala Thr Glu Glu  
50 55 60

Gln Ala Thr Glu Ser Val Ser Leu Tyr Tyr Tyr Gly Ile Lys Asp Leu  
65 70 75 80

Ala Thr Val Phe Phe Tyr Met Leu Val Ala Ile Ile Ile His Ala Val  
85 90 95

Ile Gln Glu Tyr Met Leu Asp Lys Ile Asn Arg Arg Met His Phe Ser  
100 105 110

Lys Thr Lys His Ser Lys Phe Asn Glu Ser Gly Gln Leu Ser Ala Phe  
115 120 125

Tyr Leu Phe Ala Cys Val Trp Gly Thr Phe Ile Leu Ile Ser Glu Asn  
130 135 140

Tyr Ile Ser Asp Pro Thr Ile Leu Trp Arg Ala Tyr Pro His Asn Leu  
145 150 155 160

Met Thr Phe Gln Met Lys Phe Phe Tyr Ile Ser Gln Leu Ala Tyr Trp  
165 170 175

Leu His Ala Phe Pro Glu Leu Tyr Phe Gln Lys Thr Lys Lys Glu Asp  
12

004050"02420860

180	185	190
Ile Pro Arg Gln Leu Val Tyr 195	Ile Gly Leu Tyr Leu 200	Phe His Ile Ala 205
Gly Ala Tyr Leu Leu Asn 210	Leu Asn His Leu Gly 215	Leu Val Leu Leu Val 220
Leu His Tyr Phe Val 225	Glu Phe Leu Phe His 230	Ile Ser Arg Leu Phe Tyr 235 240
Phe Ser Asn Glu Lys Tyr Gln Lys 245	Gly Phe Ser Leu Trp Ala Val 250 255	
Phe Val Leu Gly Arg Leu Leu Thr 260	Leu Ile Leu Ser Val Leu Thr Val 265 270	
Gly Phe Gly Leu Ala Arg Ala 275	Glu Asn Gln Lys Leu Asp Phe Ser Thr 280 285	
Gly Asn Phe Asn Val Leu Ala Val 290	Arg Ile Ala Val Leu Ala Ser Ile 295 300	
Cys Val Thr Gln Ala Phe Met Met Trp Lys 305	Phe Ile Asn Phe Gln Leu 310 315 320	
Arg Arg Trp Arg Glu His Ser Ala Phe 325	Gln Ala Pro Ala Val Lys Lys 330 335	
Lys Pro Thr Val Thr Lys Gly Arg Ser Ser Lys Lys Gly Thr Glu Asn 340		350
Gly Val Asn Gly Thr Leu Thr Ser Asn Val Ala Asp Ser Pro Arg Asn 355		365
Lys Lys Glu Lys Ser Ser 370		

<210> 7  
 <211> 24  
 <212> DNA  
 <213> Homo sapiens



ttc	agc	cag	gag	ttc	gtc	atc	cac	aac	cat	gcg	gac	atc	ggc	ttc	tgc	159
Phe	Ser	Gln	Glu	Phe	Val	Ile	His	Asn	His	Ala	Asp	Ile	Gly	Phe	Cys	
		15				20						25				
ctg	gtg	ctc	tgc	gtc	ctc	atc	ggg	ctt	atg	ttc	gag	gtc	aca	gcc	aag	207
Leu	Val	Leu	Cys	Val	Leu	Ile	Gly	Leu	Met	Phe	Glu	Val	Thr	Ala	Lys	
		30				35						40				
act	gcc	ttt	cta	ttt	att	tta	cct	cag	tat	aac	att	agc	gtg	cct	aca	255
Thr	Ala	Phe	Leu	Phe	Ile	Leu	Pro	Gln	Tyr	Asn	Ile	Ser	Val	Pro	Thr	
		45				50						55				60
gca	gac	agt	gag	acc	gtg	cac	tac	cac	tat	ggc	cct	aag	gac	ctg	gtc	303
Ala	Asp	Ser	Glu	Thr	Val	His	Tyr	His	Tyr	Gly	Pro	Lys	Asp	Leu	Val	
				65						70				75		
aca	atc	ttg	ttc	tac	atc	ttc	atc	acc	atc	atc	ttg	cat	gct	gtg	gtt	351
Thr	Ile	Leu	Phe	Tyr	Ile	Phe	Ile	Thr	Ile	Ile	Leu	His	Ala	Val	Val	
		80						85						90		
cag	gag	tac	att	tta	gat	aaa	atc	agc	aaa	cgg	ctt	cat	ctc	tcc	aaa	399
Gln	Glu	Tyr	Ile	Leu	Asp	Lys	Ile	Ser	Lys	Arg	Leu	His	Leu	Ser	Lys	
		95				100						105				
gtc	aaa	cac	agc	aag	ttc	aat	gaa	tct	gga	cag	ctg	gtc	gtc	ttt	cat	447
Val	Lys	His	Ser	Lys	Phe	Asn	Glu	Ser	Gly	Gln	Leu	Val	Val	Phe	His	
		110				115						120				
ttc	acc	tcg	gtg	att	tgg	tgc	ttc	tac	gtg	gtg	gtg	acg	gaa	gga	tac	495
Phe	Thr	Ser	Val	Ile	Trp	Cys	Phe	Tyr	Val	Val	Val	Thr	Glu	Gly	Tyr	
		125				130						135				140
tta	aca	aac	cca	aga	agc	ctc	tgg	gaa	gac	tac	cgg	cat	gtg	cac	ctc	543
Leu	Thr	Asn	Pro	Arg	Ser	Leu	Trp	Glu	Asp	Tyr	Pro	His	Val	His	Leu	
				145						150				155		
ccc	ttc	cag	gtg	aag	ttt	ttc	tac	cta	tgc	cag	ctg	gcc	tac	tgg	ctg	591
Pro	Phe	Gln	Val	Lys	Phe	Phe	Tyr	Leu	Cys	Gln	Leu	Ala	Tyr	Trp	Leu	
		160						165						170		
cac	gca	ctt	cct	gag	cta	tac	ttc	cag	aag	gta	cgg	aag	gag	gaa	att	639
His	Ala	Leu	Pro	Glu	Leu	Tyr	Phe	Gln	Lys	Val	Arg	Lys	Glu	Glu	Ile	
		175				180						185				
ccc	cgc	cag	ctc	cag	tat	att	tgc	ctg	tac	ctg	gtg	cat	ata	gct	gga	687
Pro	Arg	Gln	Leu	Gln	Tyr	Ile	Cys	Leu	Tyr	Leu	Val	His	Ile	Ala	Gly	
		190				195						200				
gca	tac	ctc	tta	aac	ctg	agc	cgc	ctg	ggc	ctg	atc	ttg	ctg	ctg	ctg	735
Ala	Tyr	Leu	Leu	Asn	Leu	Ser	Arg	Leu	Gly	Leu	Ile	Leu	Leu	Leu	Leu	
		205				210						215				220
cag	tac	tca	act	gag	ttc	ctc	ttc	cac	acg	gct	aga	ctc	ttc	tac	ttt	783
Gln	Tyr	Ser	Thr	Glu	Phe	Leu	Phe	His	Thr	Ala	Arg					

gca gat gaa aac aac gag aaa ctg ttc agt gcc tgg gct gct gtt ttt	831
Ala Asp Glu Asn Asn Glu Lys Leu Phe Ser Ala Trp Ala Ala Val Phe	
240 245 250	
ggg gtt acc cgc ctc ttc atc ctc acc ctt gcc gtg ctg gcc att ggc	879
Gly Val Thr Arg Leu Phe Ile Leu Thr Leu Ala Val Leu Ala Ile Gly	
255 260 265	
ttt gga ctg gct cgc atg gaa aac cag gca ttt gat ccc gag aaa ggg	927
Phe Gly Leu Ala Arg Met Glu Asn Gln Ala Phe Asp Pro Glu Lys Gly	
270 275 280	
aac ttc aac act ttg ttt tgc agg ctc tgc gtg ctg ctg ctg gtg tgt	975
Asn Phe Asn Thr Leu Phe Cys Arg Leu Cys Val Leu Leu Leu Val Cys	
285 290 295 300	
gcc gcc cag gcc tgg ctc atg tgg cgc ttc atc cac tcc cag ctg cgg	1023
Ala Ala Gln Ala Trp Leu Met Trp Arg Phe Ile His Ser Gln Leu Arg	
305 310 315	
cac tgg cgg gaa tac tgg aat gag cag agt gca aag cgg aga gtc cca	1071
His Trp Arg Glu Tyr Trp Asn Glu Gln Ser Ala Lys Arg Arg Val Pro	
320 325 330	
gcc aca ccc aga cta cca gcc agg ctc atc aag agg gaa tct ggt tac	1119
Ala Thr Pro Arg Leu Pro Ala Arg Leu Ile Lys Arg Glu Ser Gly Tyr	
335 340 345	
cat gaa aat gga gtg gtg aag gca gag aac gga acc tcc cca cgg act	1167
His Glu Asn Gly Val Val Lys Ala Glu Asn Gly Thr Ser Pro Arg Thr	
350 355 360	
aag aaa ctc aag tct ccc taa ggccaaagtg ctaagaacag gaatcctctt	1218
Lys Lys Leu Lys Ser Pro	
365 370	
gggtggggggcc gagcaggggg caaggagccc agggcccctc cctgcoctoct ccttcctgcc	1278
tgtgatgtctc cgtctcaaac agccgaaacc tgtcttgcaa tgggggggagg gggcgtttcg	1338
ctttcctttct tcttggtctc ctcttattct tccacaaacc attctcaata aagccaaaaa	1398
tctttctctt tctccccctc aggccacctc ctgtcctcac tctgttctgtg tgetggcttt	1458
tctggaacgc caggcgccca tggctggcac ctttctgctt gctctgtttc ttgccttatg	1518
getgetgett ttctttttta ctctctatct tcacctatc ttgcaatttt tctgtctgat	1578
ttttacaatg ggagggggagc taagattgca gtcctgtcct tcgggtcccc agggcctgcc	1638
ggtcagaagc ctgggggctgg taggcccttg gtggctctca tgtggatggg caagaagaga	1698
gcggccatct cggatcataa tctccttggt gctgattaac tgacgagata tatgattcca	1758
gttctgcatg taccatcttg aggcacagca gccactgtc gttgtaaatg ccaaggcatt	1818

tggttttggg	acgtgacaac	tcaatccaga	aggatggtgt	gaactcgggt	gggtcccgtg	1878
actcgagctc	ctaccagtgg	ctggccgcgg	attggaagcc	agcctgctgt	cgctctgtgg	1938
ggaggacatg	tcttcccact	gcttagagcg	agagcagagc	aaactgcgca	gcaggcacct	1998
ccagaaaggt	aatggtggca	gaaccacag	tggagtcgac	ctaggccttt	ctccagcagt	2058
cccagtcgcc	attgcttttt	cagccattca	caagcattca	aaaccaaacc	aaacagcagt	2118
tcatatacct	gcctgagata	ggctggtcct	cacctccaga	gccagccagc	cccgtcaggg	2178
gccaaactta	ctaccttgac	ttcatctcta	gctgcagaaa	cactaagtct	caagggcttc	2238
agccccatgc	tgggtccctg	gtgttcaggg	agggtcactt	ggaccgctgt	tcattctggcc	2298
gcccttggtg	agtgttcttt	ggaattgtcg	ttttttgagc	acaactacag	catttttagac	2358
tgcatgaaac	catgactgac	tgagagtcac	tctctgggta	gatgataggc	gcctttcttg	2418
ccccttccct	cacagattct	ttccccctcc	ctccacctga	agagaaggcc	tccaagtcct	2478
tttggtgcct	tgtgaggact	tttagaaggg	gcgttcagct	ttaaaaagcc	ggtcctaatt	2538
acggccggac	gcagtagctt	acgcctgtta	tccagcact	ttgggaggtc	gaggtgggca	2598
gatcacctga	ggttaggagt	tcaagaccag	cctggccaac	atggtgaaac	cccatctcta	2658
ctaaaaatac	aaaaaattag	gtgtagtggc	aggcacctgt	aatcccagct	actcgggagg	2718
ctgaggcagg	agaatcgctt	gaacctagaa	ggtggagggt	gcagtgagcg	gagattgtac	2778
catggcactc	cagcctggac	aacaagagcg	aaattctgtc	taaaaaaaca	aaagtcccaa	2838
ttaagaacct	ccgaactctg	ttttgaggca	aaggggagta	gttcttggtg	ggtgcaggaa	2898
tagtagtgtc	atttggaata	ctggtcattt	ttctgacatc	acagtagaaa	ccaaaccttg	2958
gatttagatt	caaaaggggg	gaaatgggtc	ttttcatcaa	ggcaactccc	cttctccaag	3018
tcacttacat	catagataaa	ttttagcttc	ccagtaactg	agggatttgt	ttcctaacgc	3078
cattggaggc	cttcatccct	ctctacgata	aggttgcaga	aatgggaaga	gctacccgtg	3138
gttgcttttg	attaccctta	ggaagtgaga	cagtgttttt	gaaaatatgt	atttctccca	3198
tttctccctc	tccttccctg	acaattctct	gggctgcaca	gcagaaaagt	tggtaaaagg	3258
gcagtttggg	ttcaacacag	cagacctgat	atgggatccc	ttagccactt	tagtcaaaca	3318
gccctgacag	agtctataat	tgagttcagg	ccccccacct	tgctaataaa	ctgcaaatac	3378
catgttcagc	cagcagcctc	ctaagcccac	cttctctccc	cattagagaa	cacctatcct	3438
agggtgctctc	caggctgtgt	cattggcagg	gcttcacatg	caggaggcct	ctctcagggt	3498

agtccagggtt aaactgttga gttgtggcctt caacagatat gtatggcatg ctgggatgtg	3558
ccagggtgcct gcgttgtgcc agttgctgga gaggtagtgt gagcagagca gctgaaatct	3618
tgccatcaag caaccctcat tctcatgcct gtaggtttcc attgctctgt cccaggacac	3678
ttgcgtgcc a gagacgccac aacttcatgt cctgtctct tgcaggctcc ccgtgctgcc	3738
agtacttcat gccttggatg tggccccacc agcccagtgg ctgggggtcag cttaggctct	3798
gcttcccagt ggacgggtgt gctaagggtt tattttatgt aaaaaaaaaa aaaaaacaaa	3858
aaaaaacct gagaccatga gtggggctgg catcttgcca gcctgggctt cagggatgtt	3918
tggggggggt ggtagaggg tagttgtagg gtactttgtc accccctcc cctgccacc	3978
ctccctggca cgtttatttc acagcagagc caagtctgtg gcaggttgac acagactgtg	4038
ttgccagagc tgaaataatt ccacttcac ctagagcgt gttgggctag cttgttctaa	4098
ttttggccac tttggctgtt ttcttcagtt ttatgcattc tctcctgcc caaagtgcc	4158
agccatttgt gaaggctctg ccagacacct ccaagcttga gagctcagca ccatgcacca	4218
agagcaggag aaaagacgta aacctacccc agcaactgtg gcctctcgac agccctggct	4278
aactaactta catttgtggg gaagccaaca gacacagcag gaggagaggg aggtggcgct	4338
ggtggaccaa ggatctgtgc taccgctcc cctccttga ggtgcagtga tgatgggagt	4398
tatttttacc atccgggcgc tgatagctgc actattaata aattgcatgt gttccttttg	4458
aaggtagggg atggttctgg gtgagagggg agcaggctga gccggcgggg gatctgctgt	4518
cctccctttt gagtcagttc taatcccatg tgtgtctggg ccaccagacc gaaatggttg	4578
ctgagaaaact tgtctgttca tgtcccaagg cataacttcc caacatttaa gaaaccccaa	4638
tagacacctc tgccctggcc acgttcacag atccttctct tgaccggaaa cctgggacc	4698
ctaagaaccc ctgaagcttg ggtgggtgt gtgcttctgg ggtctctttt gggacctct	4758
ttgtcagtac cccttctttt ttctaagcag ctaataagag gttgggtgaa agagtgcac	4818
tcctcccagg attccacaac aaaattctta tottccatgg atgctttaat tggaagtggg	4878
ttgccgacct ccttgtgcct agaaaaggcc tttgcttggg tttcctttgt atgcttcagc	4938
cttctagtt ggtttttcta ggctggtgt gagaggtagg gaagtctgca cataactaat	4998
tcttttgctt aagggcctat ggcacaagt cacaaacttc aattcttgat gttctaagct	5058
ctctcctcta acagagggag tgctgaaagc ttttgagtca agacaatgga gtgctcttcc	5118
tcctcactc tgccttccga gcttatggtt ccttttctca ggagaggatt ttcaggatta	5178

ttggaggatt aggtcattgt cagatgactg gaaaacctaa ataggatctc tctccagctc	5238
aaggttgtcc cagtgaggaa gactttacca acttctcaact ctaccccaact actcacatga	5298
gtgttagctc caccttgcaa aggctgaaga ccagttctcc ccagtgaag ctgcctcatt	5358
cttttatgga gttccctgga gtggcagagc tataaagacg agcattggga tttgcagtct	5418
ccatgtagcc tttcgtgctt ggcaaccct gtagactttt tgtcccaagc agattgcgtg	5478
cgtgcgcctg tgtgtgagaa taagtgcctt actttgctgt gtggttttca acttgtactc	5538
cgtggccagc cccagttgc cagggctcga cggcagccaa ggacaccata cctcagtata	5598
gttatatata aaatggacac ggattgtgac agtttcaccc catttgtttc taaccccgct	5658
gcccaggatt agggctctgt gtgtgttctg ttttgttttt ggtttctccc ttgtgtcagt	5718
tctcttctgg ccagctggg tggctgtgga agtctgtgag gtggcccaac cacaagcata	5778
cctattaaga gaagcccaga gttccagcc cccacttcga aaactctcct ctggccccac	5838
atagcaaact cttctccgt tattttcccc accccagat tttttttaa aggccactt	5898
gccataacct cttttggtct attttgcttc ccattcagcc caaagtttat atgataaagg	5958
tgtttacttt tacttcccag tctccaagt ctaacacata aacacatata tgtctgactg	6018
ttgcagaact gttcgagctc ctaattcagt gttaccttgt tttagtcgca gcaaccctct	6078
cccctacccc ttgccgccc acgtttttct cactcttccg ggttgtgcaa taactctccc	6138
agccagtggc ctttccaca gcctttctgt cccttaaaac acctgcaact gggggagaaa	6198
tgggacccat gggaggggga gtcacatcc cttacacaag aaatagccac tttccttttg	6258
ttgtcattct tgtgatcctg ggtgggttct tgtggcactc ttttagaaca tgtagcatca	6318
tcttagaggt ctatttttaa aaaatgtgtt gaagaggaaa aaaccattct cacgatggg	6378
cttaagtcac tgtccaggaa taagattgac gtggtgccc tgacatcacc gtcactctgc	6438
ctaaaagcac tctagagcta cttgttcacg tggagaggaa ggatattttg cgaagcaaca	6498
gccgcagggt gagagccctg ttcacctgat agggctctagc tgtgacagta aatataatac	6558
cgctgtttcc ttgggtacag atttgagtgt tcatgtgatg agactgtaaa cctcattttt	6618
cggttcctct gtttaaaaaa acatctgaag gatgaactaa ggctgctggg gccctgagca	6678
actgataatg caaatgtgga caaagtgtct gttttctact ctagcctgtt catatggacc	6738
aaatttcaac aaggaactca aggaaaattt gtacctgccg tatttatgct ttcagtataa	6798
aaagggttgg ggggaggggt gtctttttgc ttttggtgaa ctttttttca aaatcatttt	6858



tccactgttt ctgtctgggt ttaaaacaaa ttacagtttt gtatggattt tttaaatagta 6918  
cattttggaa caaatgatca aatattttct gaaataacaa taaaaggcag aaaatt 6974

<210> 13  
<211> 370  
<212> PRT  
<213> Homo sapiens

<400> 13

Met Ala Phe Arg Arg Arg Thr Lys Ser Tyr Pro Leu Phe Ser Gln Glu  
1 5 10 15

Phe Val Ile His Asn His Ala Asp Ile Gly Phe Cys Leu Val Leu Cys  
20 25 30

Val Leu Ile Gly Leu Met Phe Glu Val Thr Ala Lys Thr Ala Phe Leu  
35 40 45

Phe Ile Leu Pro Gln Tyr Asn Ile Ser Val Pro Thr Ala Asp Ser Glu  
50 55 60

Thr Val His Tyr His Tyr Gly Pro Lys Asp Leu Val Thr Ile Leu Phe  
65 70 75 80

Tyr Ile Phe Ile Thr Ile Ile Leu His Ala Val Val Gln Glu Tyr Ile  
85 90 95

Leu Asp Lys Ile Ser Lys Arg Leu His Leu Ser Lys Val Lys His Ser  
100 105 110

Lys Phe Asn Glu Ser Gly Gln Leu Val Val Phe His Phe Thr Ser Val  
115 120 125

Ile Trp Cys Phe Tyr Val Val Val Thr Glu Gly Tyr Leu Thr Asn Pro  
130 135 140

Arg Ser Leu Trp Glu Asp Tyr Pro His Val His Leu Pro Phe Gln Val  
145 150 155 160

Lys Phe Phe Tyr Leu Cys Gln Leu Ala Tyr Trp Leu His Ala Leu Pro  
165 170 175



157 PRTS

## DESCRIPTION

### NOVEL PROTEIN WAR-1 AND GENE THEREOF

#### 5 TECHNICAL FIELD

09807470-05401  
The present invention relates to a novel protein referred to as WAR-1, and the gene thereof. More particularly, it relates to a novel protein, WAR-1, having an inhibitory effect on cancer cell proliferations, the gene encoding WAR-1, an antibody directed to WAR-1, and uses of these substances in diagnostic and therapeutic fields.

Further, the invention relates to a composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient an endoplasmic reticulum membrane protein, a WAR-1 polypeptide, which is recognized to be expressed specifically in brain, or a gene encoding the same.

#### BACKGROUND ART

In recent years, anti-tumor agents have been developed toward various aspects, and those agents targeting replications of cells or central dogma comprising gene transcriptions and translations, as well as those agents directed to signal transmittances, differentiation, cell cycles, metabolisms, apoptosis, telomerase, or the like, have been developed. However, decisive anti-tumor agents have not been found yet, and there is a demand for anti-tumor agents based on a new mechanism.

On the other hand, it has been known that mRNAs of proteins

having a signal peptide are transported through endoplasmic reticulum (ER) membrane after the signal peptide is synthesized, and then translations occur within the ER. A factor referred to as TRAM was known as an entity responsible for the ER membrane transport (ER membrane passage) (Görlich et al., Nature, 357, 47-52, 1992).

Typically, the translation starts on binding mRNAs of proteins to be transported to the ER membrane to ribosome after being imported into the cytoplasm, and a signal sequence necessary for membrane transport is bound to a signal sequence-recognition protein.

Subsequently, the complex is bound to a receptor for the signal sequence-recognition protein to be anchored on the ER membrane. The complex between the protein to be translated and the ribosome is released from the signal sequence-recognition protein, and binds to Sec61p, while TRAM resident on the ER membrane recognizes and associates with the complex. Then, the translated protein is transported into the lumen of ER through Sec61p. Such events have been understood (Jungnickel et al., Cell, 82, 261-270, 1995). It has been also known that TRAM is not always necessary for the ER membrane transport of proteins having certain signal sequences, which constitutes an exception to the mechanism for the ER membrane transport as mentioned above (Voigt et al., J. Cell Biol., 134, 25-35, 1996).

No substance that shares a homology with TRAM responsible for the ER membrane transport with respect to the amino acid sequence and the base sequence has not been reported, except for KIAA0057 gene that had been discovered in acute myeloid leukemia

cells (Nomura et al., DNA Res., 1, 223-229,1994). Further, any information such as the relationship between the substances and the cancer has not been reported.

## DISCLOSURE OF THE INVENTION

The present invention aims to provide a novel protein, WAR-1, and the gene thereof. Specifically, it aims to provide a novel protein, WAR-1, whose structure shares a high homology with TRAM, and whose function involves an inhibitory effect on cancer cell proliferations; the gene encoding WAR-1; an antibody directed to WAR-1, and uses of these substances in diagnostic and therapeutic fields.

Further, the invention aims to provide a composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a WAR-1 polypeptide that an ER membrane protein, a portion thereof, or a gene encoding the same.

The inventors of the present application conducted the random screening for clones from the juvenile rat cDNA library. During the screening, the inventors successfully cloned the gene of a novel protein that shares a high homology with TRAM responsible for the ER membrane transport (the ER membrane passage) (Görlich et al., Nature, 357, 47-52, 1992) and with KIAA0057 (Nomura et al., DNA Res., 1, 223-229,1994). The inventors named the novel protein WAR-1. WAR-1, which structure shares a homology with TRAM as shown above had been unclear in terms of its function. Our continued study revealed that the expression of a WAR-1 gene in cancer cells inhibited their proliferation, and it has been found that WAR-1 of the present

invention has an inhibitory effect on cancer cell proliferations. Accordingly, the pharmaceutical composition comprising as an active ingredient WAR-1 of the present invention or the gene encoding WAR-1 is believed to be useful as novel anti-cancer medicaments. In view of the fact that WAR-1 of the present invention also inhibit the proliferation of sarcomas that show high malignancies, the protein is expected to have availability in the clinical field.

As a result of the studies of the expression of the WAR-1 gene in tissues and various cancer cells, it was found that although the WAR-1 gene is not normally expressed in tissues such as liver, lung, and lymphoid tissue (spleen, thymus, and leukocyte), the malignant transformation causes specific expression of the gene. Accordingly, it is believed that a partial fragment of the WAR-1 gene or an antibody directed to WAR-1 would be used in diagnosis for these cancers.

On the other hand, in view of the fact that the gene of the protein WAR-1 responsible for the membrane transport of secretory proteins into the ER is overexpressed in the adult rat brain, the present inventors studied and discovered that enhanced expression of the gene in glial cells induces increased secretion of proteins having effects on neurite extension. Further, it was found that overexpression of the gene facilitates or accelerates the secretions of diverse neurotrophic factors produced by glial cells or nerve cells themselves.

Specifically, the present inventors demonstrated that the gene of the rat type of WAR-1 (hereinafter may be abbreviated as rWAR-1) of SEQ ID: No. 1 is expressed in not only the brain but also the retina. Further, the inventors demonstrated that the gene of the human type of

WAR-1 (hereinafter may be abbreviated as hWAR-1) of SEQ ID: No. 2 is specifically expressed in not only the central nervous system but also the peripheral nervous system on the basis of the showing that the hWAR-1 gene is expressed in the whole brain and the spinal cord.

Furthermore, the inventors demonstrated that neurotrophic factors having effects on neurite extension of PC12 cells derived from rat adrenal melanocytoma are expanded in culture supernatant of human glioblastoma, T98G cells, which are infected with an adenovirus vector containing the hWAR-1 gene.

The present invention has been completed on the basis of the findings as described above.

Thus, the present invention relates to:

(1) A DNA encoding a protein selected from a group consisting of:

- (a) a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, and
- (b) a protein comprising an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein having an inhibitory effect on cancer cell proliferations;

(2) A DNA selected from a group consisting of

- (c) a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, and
- (d) a DNA that hybridizes with the DNA of the above (c) under stringent conditions, and encodes a protein having an inhibitory effect on cancer cell proliferations;

(3) The DNA of the above (2), which is cloned from chromosomal DNA libraries using all or part of the DNA of SEQ ID: No. 1 or SEQ ID: No. 3 as probe;

(4) The DNA of the above (3), which contains a promoter region;

(5) The DNA of the above (1) or (2), which is contained in the microorganism of deposit number FERM BP-6910 or FERM BP-6911;

(6) A protein obtainable by the expression of the DNA of any one of the above (1) to (5);

(7) A recombinant expression vector comprising the DNA of any one of the above (1) to (5);

(8) A recombinant adenovirus vector comprising the DNA of any one of the above (1) to (5);

(9) A transformant wherein the cell is transformed with the recombinant expression vector of the above (7) or (8);

(10) A DNA which is useful as hybridization probe or PCR primer, which is a single- or double-stranded DNA comprising all or part of the DNA of any one of the above (1) to (5), and which makes possible the specific detection of the expression of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3;

(11) The DNA of the above (10), which consists of the following sequences:

5' primer sequence; 5'-CACCTGGCTGGATCGCAGAATCGG-3' (SEQ ID: No. 7)

3' primer sequence; 5'-CTCTTTCCTCTTTGGCGGACAGTC-3' (SEQ ID: No. 8);

(12) A method for detecting the expression of a DNA comprising



the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, which comprises using the DNA of the above (10) or (11) as hybridization probe or PCR primer;

(13) An antibody that binds to the protein of the above (6);

(14) A method for detecting the expression of a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, which comprises using the antibody of the above (13);

(15) A method for diagnosing cancers, which comprising the method for the detection of the above (12) or (14);

(16) A pharmaceutical composition comprising the DNA of any one of the above (1) to (5), or the protein of the above (6) as an active ingredient;

(17) A composition for inhibiting proliferation of cancer cells, which is characterized in that the composition enhances the expression level of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3;

(18) A composition for inhibiting proliferation of cancer cells, which comprises the DNA of any one of the above (1) to (5) as an active ingredient;

(19) A composition for inhibiting proliferation of cancer cells, which comprises an adenovirus vector;

(20) A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a DNA encoding a protein selected from a group consisting of:

(a) a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, and

(b) a protein comprising an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein having a facilitatory effect on neurotrophic factor secretions;

5 (21) A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a DNA selected from a group consisting of:

(c) a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, and

10 (d) a DNA that hybridizes with the DNA of the above (c) under stringent conditions, and encodes a protein having a facilitatory effect on neurotrophic factor secretions;

(22) The composition for facilitating neurotrophic factor secretions of the above (21), which comprises as an active ingredient a DNA that is cloned from chromosomal DNA libraries using all or part of the DNA of SEQ ID: No. 1 or SEQ ID: No. 3 as probe;

(23) The composition for facilitating neurotrophic factor secretions of the above (22), which comprises as an active ingredient the DNA that contains a promoter region;

20 (24) The composition for facilitating neurotrophic factor secretions of the above (20) or (21), which comprises as an active ingredient a DNA that is contained in the microorganism of deposit number FERM BP-6910, or FERM BP-6911;

25 (25) The composition for facilitating neurotrophic factor secretions of any one of the above (20) to (24), wherein the DNA is comprised in a recombinant expression vector;

00807470-050401  
FOI050-02420860

(26) The composition for facilitating neurotrophic factor secretions of the above (25), wherein the DNA is comprised in an adenovirus vector.

(27) A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a protein selected from a group consisting of:

(a) a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, and

(b) a protein comprising an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein having a facilitatory effect on neurotrophic factor secretions;

(28) A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a protein encoded by a DNA selected from a group consisting of:

(c) a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, and

(d) a DNA that hybridizes with the DNA of the above(c) under stringent conditions, and encodes a protein having a facilitatory effect on neurotrophic factor secretions;

(29) The composition for facilitating neurotrophic factor secretions of the above (27) or (28), which comprises as an active ingredient a protein encoded by a DNA that is contained in the microorganism of deposit number FERM BP-6910, or FERM BP-6911;

(30) A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a substance for

enhancing the expression level of the DNA of SEQ ID: No. 1 or SEQ ID: No. 3 or a substance for enhancing the production level of a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4;

(31) A pharmaceutical composition for treating neurodegenerative diseases, which comprises the composition for facilitating neurotrophic factor secretions of any one of the above (20) to (30); and

(32) A method for facilitating secretion of neurotrophic factors, which comprises enhancing the expression level of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, or enhancing the production level of a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4.

In one aspect, the present invention provides a novel protein, WAR-1, and a DNA encoding WAR-1.

In the present invention, the DNA encoding the protein is not limited to particular one as long as the DNA encodes the novel protein, WAR-1, or the DNA is similar to the DNA encoding WAR-1, and encodes a protein having an inhibitory effect on cancer cell proliferations.

Specific examples include the following DNAs of (1) to (3):

1) a DNA encoding a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, or a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3;

2) a DNA encoding a protein comprising an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein having an inhibitory effect on cancer cell

proliferations; and

3) a DNA that hybridizes with a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3 under stringent conditions, and encodes a protein having an inhibitory effect on cancer cell proliferations.

These DNAs are described in a sequential manner as follows.

1) DNA encoding WAR-1

Among the DNAs as mentioned above, "DNA encoding a protein comprising the amino acid sequence of SEQ ID: No. 2", and "DNA comprising the base sequence of SEQ ID: No. 1" are those encoding rat WAR-1 of the present invention. Further, "DNA encoding a protein comprising the amino acid sequence of SEQ ID: No. 4", and "DNA comprising the base sequence of SEQ ID: No. 3" are those encoding human WAR-1 of the present invention. Such DNAs encoding rat and human WAR-1s of the present invention have been deposited as shown below.

Specifically, *E. coli* DH5 $\alpha$  (prWAR-1) that is an *E. coli* containing prWAR-1 wherein the DNA encoding rat WAR-1 of SEQ ID: No. 1 is incorporated into a vector pBluescript II, and *E. coli* DH5 $\alpha$  (phWAR-1) that is an *E. coli* containing phWAR-1 wherein the DNA encoding human WAR-1 of SEQ ID: No. 3 is incorporated into a vector pBluescript II were deposited at The National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology (1-1-3 Higashi, Tsukuba, Ibaraki, Japan) under deposit numbers FERM P-17018 and FERM P-17019, respectively (deposition date: October 6, 1998 in each case). Subsequently, they were converted to

international deposition (deposit numbers: BP-6910 and BP-6911; date of conversion to international deposition: October 7, 1999 in each case).

2) DNA encoding altered form or variant of WAR-1

Among the DNAs as mentioned above, "DNA encoding a protein comprising an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein having an inhibitory effect on cancer cell proliferations" are those DNAs that encodes a protein having an inhibitory effect on cancer cell proliferations, among DNAs encoding altered forms (altered proteins) that are artificially prepared, or variants existing in the living body.

Those skilled in the art would readily prepare DNAs encoding the altered forms as described above by, for example, methods using restriction enzymes or nucleases, the site-directed mutagenesis (W.Ito et al., Gene, 102, 67-70 (1991), or the PCR method (Molecular Cloning, 2nd Edt. Cold Spring Harbor Laboratory Press (1989)).

In this context, the number of amino acid residues to be subject to "deletion, substitution and/or addition" is in the range that deletion, substitution and/or addition can be achieved by the well-known methods such as site-directed mutagenesis as mentioned above.

Further, it is possible to prepare DNAs encoding the altered forms by exposing cells to mutagens without the genetic engineering approaches as mentioned above.

DNAs encoding the variants as mentioned above refer to those naturally occurring in the living body. Specifically, the deletion, substitution or addition of base or amino acid may be caused by

natural events such as cancers or species specificity, and DNAs encoding such naturally occurring variants are also fallen within the DNAs of the present invention as long as those DNAs encode proteins having inhibitory effects on cancer cell proliferations.

5           Determination whether or not the DNAs containing alterations as shown above encode a protein having an inhibitory effect on cancer cell proliferations may be conducted by the following procedures.

          Specifically, a candidate DNA for those of the present invention, such as the DNAs containing the alteration as mentioned above is  
10           incorporated into an expression vector, and the resultant vector is transformed into a cancer cell line. As a cancer cell line, human glioblastoma such as T98G is preferably used. Expression vectors may be either non-viral vectors or viral vectors, and are not limited to particular vectors as long as they can be expressed in cancer cell lines  
15           from human (the details about the expression vectors are described hereinafter). After transforming the recombinant vectors into the cancer cell lines, and culturing the same, the cell counts and morphologic change of the cells are examined. At this time, it is important to compare them with those of cells prepared as a control in  
20           accordance with the same procedure except for the use of an expression vector without the incorporated foreign DNAs. When observing that cell count is decreased, or morphology of the cell is changed compared to the control cells, then the candidate DNA may be  
25           determined to be one encoding a protein having an inhibitory effect on cancer cell proliferations.

          Additionally, determination whether or not an inhibitory effect

on cancer cell proliferations is exhibited may be conducted by, for example, measuring a decrease in  $^3\text{H}$ -labeled thymidine incorporation in the gene-transferred cells as mentioned above (Nagase et al., Int. J. Cancer, 65, 620-626, 1996), or measuring a decrease in tumorigenicity observed in nude mice inoculated with cancer cells that have been infected with an adenovirus having a candidate DNA as mentioned above (Cheney et al., Cancer Res., 58, 2331-2334, 1998).

3) DNA that hybridizes with the WAR-1 DNA under stringent conditions

Among the DNAs as described above, "DNA that hybridizes with a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3 under stringent conditions, and encodes a protein having an inhibitory effect on cancer cell proliferations" refers to DNAs that encode a protein having an inhibitory effect on cancer cell proliferations, among DNAs that hybridize with the rat or human WAR-1 DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3 under stringent conditions, such as

- A) a cDNA encoding WAR-1s from all vertebrates, or a cDNA encoding a partial protein of WAR-1s, and
- B) a chromosomal DNA encoding WAR-1s from all vertebrates.

In this context, "DNA that hybridizes under stringent conditions" refers to those DNAs that continue to be hybridized under such a condition that the hybridization is conducted at 42°C overnight using, as hybridization buffer, a solution of a composition of 0.1% SDS, 50% formamide, 5xSSC, 1xDenhardt reagent, and 250 µg/ml salmon sperm DNA, followed by washing with 2xSSC for an hour at room temperature, with 2xSSC, 0.1% SDS for 30 minutes at room



temperature, and then with 0.1xSSC, 0.1%SDS at 50-65°C for 30 minutes.

The DNA of the above (A) may be cloned by, for example, hybridization using as a probe all or part of the DNA of SEQ ID: No. 1 or SEQ ID: No. 3, or by PCR using as a primer a part of DNA of SEQ ID: No. 1 or SEQ ID: No. 3. Particular procedures of the preparation of cDNA library, hybridization, PCR, selection for positive colony, sequencing of the base sequence, or the like are well known, and may be conducted according to documents such as Molecular Cloning 2nd Edt., Cold Spring Harbor Laboratory Press (1989). An example for the particular cloning procedure is provided below.

The DNA of the above (A) may be isolated by a process comprising the steps (a) and (b) for example:

- a) preparing total RNA from tissues derived from a desired species or cultured cell lines, and purifying the poly(A) RNA so as to provide a cDNA library; and
- b) hybridizing the cDNA library prepared in the above (a) with a probe prepared to comprise all or part of the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, thus isolating an intended DNA.

In this context, the preparation of total RNA in the above step (a) may be conducted according to usual manners that include for example treating cells with surfactants such as SDS, NP-40, Triton-X100 or in the presence of phenol, so as to effect cytolysis. Further, the preparation of total RNA may be also conducted by means of destroying cells via physical means such as using a homogenizer, treating the cell with guanidine thiocyanate, and then precipitating

total RNA via cesium chloride density gradient centrifugation, or by means of treating the cell with guanidine thiocyanate, and with phenol in an acidic condition (acidic guanidine thiocyanate-phenol chloroform). Subsequently, affinity columns such as oligo(dT)-cellulose and polyU-cellulose bound with polyU are used to purify poly(A)RNA (mRNA) from the total RNA obtained by any one of the above methods. Alternatively, in cases that the length of the mRNA is known, or fractions based on the length of the mRNA are intended, sucrose density gradient centrifugation, agarose gel electrophoresis, gel filtration on columns, or the like may be used. Preparation of cDNA libraries from the mRNA obtained as described above may be conducted by synthesizing a single-stranded cDNA using the mRNA as a template, preparing a double-stranded cDNA, incorporating the same into suitable vectors, and transforming *E. coli* hosts with the vectors. As vectors, plasmids and  $\lambda$  phage vectors are often used. Details on the preparation of the cDNA libraries are provided below.

First, a single-stranded cDNA complementary to the mRNA is synthesized with reverse transcriptase (from avian myeloblastic leukemia virus; AMV, or from murine leukemia virus; Mo-MLV) using mRNA template and oligo (dT) primers attached with or without a suitable sequence at the end, or random primers consisting of six bases. Then, the mRNA is decomposed with alkaline conditions, and then a double-stranded cDNA is synthesized with reverse transcriptase or DNA polymerase using the single-stranded cDNA as template. Alternatively, double-stranded cDNA may be directly synthesized using RNase H and *E. coli* DNA polymerase I. In both cases, subsequently,

one of enzymes such as S1 nuclease, T4 DNA polymerase, and *E. coli* DNA polymerase (the Klenow fragment) is used to form a blunt end at both terminuses of the synthesized double-stranded cDNA. Terminal modification is conducted on the resultant blunted double-stranded cDNA by attaching chemically-synthesized DNAs such as linker and adaptor, or dG or dC strand to the cDNA with deoxy terminal transferase so as to be ready for the incorporation of the cDNA into a suitable vector. The double-stranded cDNA is incorporated into a suitable vector, and then the vector is transformed into *E. coli* to prepare cDNA libraries. In case of the transformation of *E. coli* hosts with plasmid vector incorporated with double-stranded cDNA, the transformation may be conducted on the harvested competent cells to be transferred with the DNA at logarithmic growth phase according to the procedure discussed by Hanahan (J.Mol.Biol.,166,557 (1983)). In case of the transformation of *E. coli* hosts with phage vector incorporated with double-stranded cDNA, the transformation may be conducted by introducing DNAs ligated with T4 DNA ligase into phage particle via *in vitro* packaging system, and infecting *E. coli* hosts with the phage.

In the next step (b), an intended DNA may be isolated by preparing probes consisting of all or part of the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, and hybridizing the cDNA library prepared as shown above with the probes. Probes in this context may be prepared by synthesizing a suitable partial fragment of the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3 with DNA synthesizers or by amplifying said partial fragment via PCR, and then labeling the DNA

fragment with  $^{32}\text{P}$  in accordance with conventional procedures such as nick translation and random priming labeling. Conditions for hybridization are as described above.

The DNA as mentioned above (B), namely, chromosomal DNA encoding WAR-1 may be cloned for example by hybridizing chromosomal DNA libraries prepared from tissues derived from desired species or cultured cell lines with probes having all or part of the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, or having all or part of the DNA of the above (A).

In this context, preparations of chromosomal DNAs and chromosomal DNA libraries may be conducted in accordance with usual manners. Specifically, tissues derived from desired species or cultured cell lines are processed in the presence of SDS, and RNAs and proteins that are unnecessary are decomposed with RNase and proteinase K. Then, chromosomal DNA may be purified by treatment with phenol, and subsequent ethanol precipitation or dialysis. The resultant chromosomal DNA is partially cleaved with suitable restriction enzymes, or completely digested with necessary restriction enzymes in case that the length of the fragment to be cloned is known. Fragmentation of the cleaved chromosomal DNA fragments based on the DNA length to be allowed for their incorporation into cloning vectors may be conducted by sucrose density gradient centrifugation, agarose gel electrophoresis, gel filtration on columns, or the like. The cleaved fragments are incorporated into  $\lambda$  phage vectors or cosmid vectors, and *in vitro* packaged, thus conducting the preparation of chromosomal DNA libraries with phages or cosmids.

0980740-0540  
T04050-02420860

The chromosomal DNA encoding WAR-1 may be cloned by infecting *E. coli* with the phage or cosmid library as described above, and then conducting plaque or colony hybridization in accordance with conventional procedures such as using, as probe, all or part of the WAR-1 cDNA labeled with  $^{32}\text{P}$  by nick translation and random priming labeling method (See Molecular Cloning 2nd Edt., Cold Spring Harbor Laboratory Press (1989)).

Chromosomal DNA as described above will contain a promoter region, which is responsible for gene expression regulation, and such chromosomal DNA that contains the promoter region may be readily cloned by the procedures as mentioned above.

Recombinant expression vectors containing the DNA of the present invention may be prepared by incorporating various DNAs of the present invention into expression vectors. Transformants of the present invention may be prepared by transforming host cells with the recombinant expression vectors.

The expression vectors include both non-viral and viral vectors, and are not limited to any particular species as long as they allow the DNA of the present invention to be transferred, and allow the proteins encoded by the DNA to be expressed. Non-viral vectors include expression plasmid vectors typically used in mammal cells, and are exemplified by pBK-CMV, pCAGGS, pcDNA3.1, pZeoSV, and the like. Transformation of non-viral expression vectors incorporated with the DNA of the present invention may be achieved by calcium-phosphate coprecipitation, methods for transferring DNA molecules using liposome (the liposome method, Lipofectin method, Lipofectamine

method), electroporation, microinjection, methods for transferring DNA molecules into cells along with carriers by particle guns, and the like. Host cells include HeLa, COS1, A549, 293 cells, and the like.

For the latter viral vectors, viral vectors such as adenovirus and retrovirus are typical. Specifically, DNA or RNA viruses such as avirulent retrovirus, adenovirus, adeno-associated virus, herpesvirus, vaccinia virus, poxvirus, poliovirus, Sindbis virus, Sendai virus, SV40, human immunodeficiency virus are incorporated with the gene encoding the given protein, and the recombinant viruses are infected to cells, thus being capable of transforming the gene into cells. In this context, host cells include 293, A549, and HeLa cells.

The transformants thus obtained may be continuously cultured under an appropriate condition to express and prepare proteins from the DNA of the present invention. In this context, the term "appropriate condition" refers to a condition wherein a cultivation is conducted at 37 °C under 5%CO<sub>2</sub> in a culture medium suitable for respective host cell.

From the transformants thus cultured in an appropriate condition, the proteins of the present invention as described above may be isolated and purified. In this connection, preparation of crude extract of the present proteins, and purification of the present proteins may be conducted according to, for example, methods as described in "Shinseikagakujiikkenn-koza 1, Tannpakushitsu I-bunnri, seisei, seishitsu", Japanese Biochemical Society Ed. 1990.

Specific examples of the proteins of the present invention obtainable as shown above include rat WAR-1 comprising the amino

acid sequence of SEQ ID: No. 2, and human WAR-1 comprising the amino acid sequence of SEQ ID: No. 4.

Inhibitory effect of the proteins of the present invention on cancer cell proliferations may be determined as described below. The protein of the invention is added to the culture of cancer cells such as T98G. At this time, the protein is encapsulated into liposome or bound to lipid in order to enhance the permeability across cell membrane. Alternatively, the endoplasmic reticulum-retained sequence, Lys-Asp-Glu-Leu (KDEL), is attached to the C-terminus of the protein in order to make it possible that the protein migrated to the cytoplasm be transported into the ER. Inhibitory effect of the protein of the present invention on cancer cell proliferations may be determined by examining the cell counts and morphologic change of the cancer cells after adding the protein thus treated to the culture, and culturing the same for several days. Further, Inhibitory effect of the protein of the present invention on cancer cell proliferations may be determined by examining the lowered incorporation of <sup>3</sup>H-labeled thymidine (Nagase et al., Int. J. Cancer, 65, 620-626, 1996).

Single- or double-stranded DNA comprising all or part of the DNA encoding the protein of the present invention may be used as hybridization probe or PCR primer to detect specifically the expression of the WAR-1 DNA in tissues of the living body or cultured cell lines. Such single- or double-stranded DNAs are not limited to any particular ones as long as they allow the specific detection of the WAR-1 mRNA that is a transcription product of WAR-1 DNA.

Specific examples of the detection include the two procedures

as shown below:

- 1) Analytical procedures wherein PCR is conducted on the substrate of total RNA or poly(A) RNA derived from test tissues or cultured cell lines, using as PCR primer two single-stranded DNAs (sense and antisense strands) that allow the specific detection of WAR-1 mRNA; and
- 2) Northern blotting analyses of total RNA or poly(A) RNA derived from test tissues or cultured cell lines wherein radiolabeled single-stranded or double-stranded DNAs that allow the specific detection of WAR-1 mRNA is used as probe.

The procedures for the detections (1) and (2) as described above may be conducted on the basis of text books such as Molecular Cloning 2nd Edt., Cold Spring Harbor Laboratory Press(1989). Specific examples are shown below:

The specific example of the procedure for PCR as described in the above (1) is as follows. First of all, PCR primer that allows the specific detection of WAR-1 mRNA is synthesized according to usual manners. Next, total RNA or poly(A) RNA is prepared as describe above from test tissue or cultured cell lines, and single-stranded DNA is prepared using the RNA as template with reverse transcriptase such as MMTV-RT. Subsequently, the PCR primer as previously prepared is added, and PCR reaction is routinely conducted. Conditions for PCR reaction include, for example, 35 cycles comprising 95°C for one minute, 60°C for one minute, and 72°C for two minutes, and subsequent heating at 72°C for 10 minutes. Electrophoresis of the PCR reactions on agarose gel at various concentrations enables the detection of presence or absence of the expression of WAR-1 mRNA.



The detection of WAR-1 mRNA may be conducted by *in situ* PCR method (Fernandez et al., Mol. Carcinog, 20, 317-326, 1997).

The specific example of the Northern blotting analyses as described in the above (2) is as follows. First of all, single- or double-stranded DNAs that allow the specific detection of WAR-1 mRNA is radiolabeled to prepare probes. Double-stranded DNA may be prepared by, for example, labeling the PCR reactions prepared in the procedure (1) as mentioned above with  $^{32}\text{P}$  in accordance with nick translation and random priming labeling. Next, total RNA or poly(A) RNA is prepared from test tissues or cultured cell lines in accordance with a similar procedure to that of the above, and formaldehyde gel electrophoresis and blotting to the nylon membrane are routinely conducted. Hybridization of the probe as previously prepared with this membrane permits the detection of presence or absence of the expression of the WAR-1 mRNA. Conditions for the hybridization wherein double-stranded DNA is used as probe include such conditions that the hybridization is conducted at 42°C for 16-45 hours using a solution of a composition of 45%(v/v) formamide, 5xSSPE, 2xDenhardt solution, 0.5%SDS, and 20 µg/ml salmon sperm DNA, followed by washing a few times with 2xSSPE and 0.5%SDS for 10 minutes at room temperature, and then a few times with 2xSSPE, 0.5% SDS at 65°C for 20 minutes.

Specific examples of single- or double-stranded DNAs as used in the detection shown above include the following.

When selecting portion to be used as probe or primer specific for human WAR-1, it is important to select regions having a low

homology between human TRAM and human WAR-1, consulting Figure 1 that gives comparison of the base sequences of human WAR-1 (hWAR-1) and rat WAR-1 (rWAR-1) with those of human TRAM (hTRAM) that is a known factor responsible for the ER transport and human KIAA0057 wherein the black frame shows portions sharing a homology. At this time, in order to discriminate between DNA fragments amplified from both cDNAs, the region containing deletion of partial base sequence in one of the sequences is preferably amplified. Primers specifically recognizing each of the cDNAs preferably share no homology each other in the whole primer sequences, and it is understood that the specificity would be enhanced when the sequences in the vicinity of the 3'-terminus are diversified largely each other to prohibit the elongation reaction of cDNA not intended to be amplified from proceeding. Further, design to differentiate base sequences at the 3'-terminus each other is effective for the specific amplification. Primer analysis using programs such as a software Oligo of National Biosciences may be also utilized.

On the basis of portions selected as shown above, probes of the above (1) or primers of the above (2) may be prepared in accordance with usual manners.

One example may be a single-stranded DNA of the following sequence, or a double-stranded DNA amplified by PCR reactions as shown above using the single stranded DNA as primer:

5' primer sequence; 5'-CACCTGGCTGGATCGCAGAATCGG-3' (SEQ ID:

No. 7)

3' primer sequence; 5'-CTCTTTCCTCTTTGGCGGACAGTC-3' (SEQ ID:

No. 8).

SEQ ID: No. 7 corresponds to the sense strand of the sequence at positions 823-846 in hWAR-1 DNA of Figure 1, whereas SEQ ID: No. 8 corresponds to antisense strand of the sequence at positions 1093-1116 in hWAR-1 DNA of Figure 1.

The single-stranded DNA or double-stranded DNA as shown above may be used as PCR primer used in the above detection (1) or hybridization probe used in the above detection (2) to detect the specific expression of WAR-1 DNA. See Example 4 for the details. Such primer and probe may include sequences derived from native WAR-1 as well as DNA sequences containing modifications such as substitution, deletion, and addition as long as the latter allows the specific detection of WAR-1 mRNA that is a transcription product of WAR-1 DNA.

The method for detecting the WAR-1 DNA expression may be specifically used in applications to diagnosis for diseases and in examinations such as *in situ* hybridization.

As mentioned in the above description for "means for dissolving the problems", it was found that although the WAR-1 gene is not normally expressed in tissues such as liver, lung, and lymphoid tissue (spleen, thymus, and leukocyte), the malignant transformation of these tissues causes specific expression of the gene. Accordingly, the detection of the WAR-1 mRNA in cancer tissues or cancer cells derived from patients using the PCR primer or the hybridization probe specific for WAR-1 as shown above leads to diagnosis of cancers.

In the present invention, "antibody" means an antibody binding to the proteins of the present invention as shown above. Such

antibodies are easily prepared, for example, according to methods described in "Antibodies: A Laboratory Manual", Lane, H. D. *et al.* eds., Cold Spring Harbor Laboratory Press (1989) and Shin-Saibokogaku-jikken-purotokoru, Shujun-sha (1993). Specifically, antibodies that bind to the proteins of the present invention may be prepared using the protein or parts thereof according to the present invention to appropriately immunize an animal in usual manners.

In this context, the proteins of the present invention used as immunological antigens may be obtained by introducing the recombinant expression vectors containing the DNA of the invention into *E. coli* or cultured cell lines, preparing a large amount of the polypeptides from the transformants, and purifying the same. Additionally, peptides comprising a partial amino acid sequence of the protein of the invention may be synthesized, and conjugated to BSA, KLH, or the like, to give immunological antigens.

Species to be immunized include any species such as rabbit, mouse, rat, chicken, bovine, donkey, ovine and horse, and may be a polyclonal or monoclonal antibody as long as those antibodies recognize the proteins of the present invention.

Such antibodies are used in detection for the expression of the WAR-1 protein, or isolation of the protein. Specifically, they may be used in an affinity chromatography, a screening for cDNA library, an immunological diagnosis, and the like.

Immunological diagnosis for cancers may be conducted using the antibodies of the present invention. Specifically, the antibodies of the invention enable to detect the cancer tissues or cells producing

WAR-1, and can be applied to the diagnosis for cancers. Specific detection methods include a fluorescent antibody method, Western blotting, immunoprecipitation, and immunohistological staining. A fluorescent antibody method among them may be conducted in accordance with specific procedures described in Samoszuk et al., Am. J. Clin. Pathol., 109, 205-210, 1998 or Bernardini et al., Tumori., 83, 673-678, 1997.

A DNA or a protein of the present invention may be comprised as an active ingredient in pharmaceutical compositions. The protein of the invention has an inhibitory effect on cancer cell proliferations, as mentioned above. Accordingly, either administration of the pharmaceutical composition comprising the DNA of the invention as an active ingredient to cancer patients, said composition being used as gene therapy agents to effect gene expressions in living bodies, or administration of the pharmaceutical composition comprising the protein of the invention as an active ingredient to cancer patients enables cancer cell proliferation to be inhibited, and makes cancer therapy possible. Not only administration of the DNA or the protein of the present invention, but also administration of a factor or a compound that induces the expression of the WAR-1 gene or protein occurring in living bodies provides the above inhibitory effects on cancer cell proliferations. Accordingly, pharmaceutical compositions for inhibiting proliferation of cancer cells that contains, as an active ingredient, factors or compounds inducing the expression of the WAR-1 gene, or factors or compounds inducing the expression of the WAR-1 protein are also fallen within the scope of the present invention.

FOI050-02420360

The pharmaceutical composition comprising as an active ingredient a protein of the present invention may be administered along with an adjuvant, or in a particulate dosage form. Specific examples of dosage form include liposomal preparations, particulate preparations in which the ingredient is bound to beads having a diameter of several  $\mu\text{m}$ , or preparations in which the ingredient is attached to lipids. The administration may be achieved in manners of sustained-release minipellet formulations. In order to transport a protein expected to occur in the ER selectively into the ER wherein the protein functions, Lys-Asp-Glu-Leu (KDEL) sequence residing at the C-terminus of proteins known to be retained in the ER may be attached to the protein at the C-terminus, similarly to the protein of the invention. It has been known that proteins having KDEL sequence at the C-terminus are bind to receptor proteins residing at Golgi body and the ER, and reverse-transported from Golgi body to the ER (Majoul et al., J. Cell Biol., 133, 777-789, 1996). Further, attachment of certain glycopeptides or carbohydrate chains to the protein or binding of biotin provides selectivity in transition to tissues. Specifically, polypeptides may be modified with asialoglycoprotein (Merwin et al., Bioconjug Chem., 5, 612-620, 1994) or galactose (Chen et al., Hum Gene Ther., 5, 429-435, 1994) to effect protein accumulation specifically at hepatocytes. Similarly, proteins binding to a protein expressed in certain tissues or cells may be biotinylated to form a complex between the biotinylated protein and avidin, thus enhancing an ability to transit to tissues (Saito et al., Proc. Natl. Acad. Sci. USA, 92, 10227-10231, 1995, Pardridge et al., Pharm. Res., 11, 738-746, 1994).

Although the dose to be administered may be adjusted as appropriate depending on, for example, the property of cancer cells, the age and the body weight of the particular patient, the dose is usually 0.001 mg/kg/administration to 1000 mg/kg/administration. It is preferred to administer such dose every day at the initial stage, and subsequently, every several days to every several months. Dosage form may be modified, and intra-arterial injection, intravenous injection, intramuscular injection, and topical injection to cancer tissues are possible.

Compositions for gene therapy comprising the DNA of the present invention achieve production of the WAR-1 protein in a large amount within the cells, and, against the cancer cells, enable to inhibit the proliferation of cancer cells.

When the composition for gene therapy comprising the DNA of the present invention is introduced into cancer cells, the dosage form may be classified into two types, namely, the case wherein non-viral vectors are used and the case wherein viral vectors are used.

The details of procedures for allowing a DNA of the invention to act within the cells are shown below.

A. When non-viral vectors are used

Transformations of recombinant expression vectors into cells may be achieved by introducing the DNA of the invention into gene-expression vectors, and transferring the DNA molecules into the cells according to calcium-phosphate coprecipitation, methods for transferring DNA molecules using liposome (the liposome method, Lipofectin method, Lipofectamine method), electroporation,

microinjection, methods for transferring DNA molecules into cells along with carriers by particle guns, and the like. In this context, expression vectors used include pBK-CMV, pCAGGS, pcDNA3.1, and pZeoSV.

B. When viral vectors are used

5 In this case, methods wherein viral vectors such as adenovirus and retrovirus are used are typical. Specifically, DNA or RNA viruses such as avirulent retrovirus, adenovirus, adeno-associated virus, herpesvirus, vaccinia virus, poxvirus, poliovirus, Sindbis virus, Sendai virus, SV40, human immunodeficiency virus are incorporated with the  
10 DNA of the invention, and the recombinant viruses are infected to cells, thus being capable of transforming the gene into cells.

Among the viral vectors, adenovirus vector system is preferably used since adenovirus is known to be extremely higher than other viral vectors in terms of infection efficiency.

15 Any one of the above methods may be used to introduce the gene encoding WAR-1 into cancer cells. In gene therapy using non-viral vectors, it is preferred to target the gene to the vicinity of an affected area by topical administration, combination with dosage form for enhancing ability to transit tissues, or the like, whereas, in gene  
20 therapy using viral vectors, topical administration is not necessary, and intravenous administration may be applicable. Dosage form may be formulations such as liquid formulation, and may be supplemented with conventional carriers if necessary. To facilitate the transfer of the gene into the vicinity of an affected area, sustained-release  
25 formulations may be applicable.

In order to allow a gene of the present invention to act as a



medicine in practice, one can use an *in vivo* method in which DNA is directly introduced into the body, or an *ex vivo* method in which certain cells are removed from human body, and after transferring the DNA into said cells extracorporeally, the cells are reintroduced into the body (Nikkei-Science, April, 1994, pp. 20-45; Gekkan-Yakuji, 36(1), 23-48 (1994); Jikkenn-Igaku-Zokan, 12(15), 1994). An *in vivo* method is more preferred in the present invention.

Although the amount of a DNA of the present invention in the formulations may vary appropriately depending on the disease to be treated, the age and weight of the patient, and the like, it is typical to administer 0.0001-100 mg, preferably 0.001-10 mg, of a DNA of the present invention every several days to every several months.

In the second aspect, the present invention provides a composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a WAR-1 protein, or a DNA encoding WAR-1.

The term "composition for facilitating neurotrophic factor secretions" means a pharmaceutical composition having a facilitatory effect on neurotrophic factor secretions from nerve cells that are contacted to said composition. The term "neurotrophic factor(s)" is a common name of proteins having physiological actions such as neuronal survival and maintenance, and neural differentiation, like nerve growth factor (NGF) that was discovered in 1950.

DNAs encoding the proteins of the present invention include, but not limited to, a DNA encoding WAR-1 protein, and a DNA similar to the DNA encoding WAR-1, which encodes a protein having a

facilitatory effect on neurotrophic factor secretions. Specific examples are DNAs of the following (1) to (3).

- 1) a DNA encoding a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, or a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3;
- 2) a DNA encoding a protein comprising an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein having a facilitatory effect on neurotrophic factor secretions; and
- 3) a DNA that hybridizes with a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3 under stringent conditions, and encodes a protein having a facilitatory effect on neurotrophic factor secretions.

The details of these DNAs are as shown above except that biological activity of a protein encoded by the DNA is a facilitatory effect on neurotrophic factor secretions rather than an inhibitory effect on cancer cell proliferations.

Determination whether or not DNAs encode a protein having a facilitatory effect on neurotrophic factor secretions may be conducted by examining a neurotrophic factor expressed in cells introduced with the DNAs, or by examining a neurotrophic factor extracellularly released. For example, the following procedures may be used in the determination.

For biochemical analysis, Western blotting, ELISA and RIA wherein antibodies that recognize diverse neurotrophic factors are used

are convenient. Also, electron microscope techniques may be combined with immunohistochemical staining using such antibodies to check the accumulation of neurotrophic factors in the ER. This approach is based on presumption that proteins freshly translated in the cytoplasm stably inhabit in the ER wherein proteinases are in low levels when the proteins are bound to signal recognition proteins or WAR-1 to form a rough ER, but otherwise are subjected to decomposition by proteinases that exist in a large level in the cytoplasm.

Additionally, biologically analytic approach may be used to examine if neurotrophic factors having neurite extension effects are accumulated in the culture supernatant of transformants receiving gene transfer. Specifically, the culture supernatant of transformants is contacted to PC12 cells derived from rat adrenal melanocytoma that are recognized to extend their neurite by the supplement with diverse neurotrophic factors, and then determination is conducted if the accumulation level of neurotrophic factors having neurite extension effects in the transformants is higher than that of control. PC12 cells are available from Dainippon Pharmaceutical Co. Ltd. or Rikenn GeneBank. For the first choice, the biological analysis is more preferred.

In the case that neurite extension effects are recognized, biochemical analysis is required for the next step to examine which neurotrophic factors increase. At this time, many antibodies are necessary to detect diverse neurotrophic factors, but, in this case, there is any possibility to detect unknown factors.

In order to confirm if unknown factors exist, the culture supernatant may be treated with neutralizing antibodies that bind to neurotrophic factors or receptors for them to cause disappearance of their activities, thus observing the lowered effect of neurite extension.

5 When significant neurite extension effects are remained even in the presence of neutralizing antibodies, it may show the presence of unknown neurotrophic factors.

10 Additionally, cells transferred with a gene for certain neurotrophic factor or cells known to release certain neurotrophic factors may be used in a method for checking that neurotrophic factor secretion is facilitated by enhancing the expression level of the WAR-1 gene. More conveniently, it is preferred that the latter cells, especially cell lines from cancers, are used.

15 As cells releasing certain neurotrophic factors, T93G cells that are glioblastoma derived from human astrocyte may be used. T93G is available from Dainippon Pharmaceutical Co. Ltd. Diverse factors such as NGF (Emmett et al., Neurochem. Int., 30, 465-474, 1997), TGF- $\beta$ 1,  $\beta$ 2 (Naganuma et al., Neurol Med Chir(Tokyo) , 36,789-795, 1996), PDGF-B (Potopova et al., Int. J. Cancer, 66,669-677, 1996),  
20 bFGF (Takahashi et al., FEBS Lett., 288, 65-71, 1991), IGF-1 (Ambrose et al., J. Cell Physiol., 159,92-100,1994) are known as neurotrophic factors released from T93G cell. Neurotrophic factor secretions facilitated by the increased production of WAR-1 protein have been demonstrated by biological analysis wherein WAR-1 gene is transferred  
25 and expressed in T93G cells that is recognized to release diverse neurotrophic factors as shown above, and wherein the neurite

extension effect of culture supernatant of the resultant transformants on PC12 cells is used as indicator.

In accordance with the above procedures, proteins may be expressed and prepared starting from the DNA of the invention by culturing continuously the cells transformed with a DNA of the invention under an appropriate condition. In this context, the term "appropriate condition" refers to a condition wherein a cultivation is conducted at 37 °C under 5%CO<sub>2</sub> in a culture medium suitable for respective host cell.

From the transformants thus cultured in an appropriate condition, the proteins of the present invention as described above may be isolated and purified. In this connection, preparation of a crude extract of the present proteins, and purification of the present proteins may be conducted according to, for example, methods as described in "Shinseikagakujikkenn-koza 1, Tannpakushitsu I-bunnri, seisei, seishitsu", Japanese Biochemical Society Ed. 1990.

Specific examples of the proteins of the present invention obtainable as shown above include rat WAR-1 comprising the amino acid sequence of SEQ ID: No. 2, and human WAR-1 comprising the amino acid sequence of SEQ ID: No. 4.

Facilitatory effect of the proteins of the present invention on neurotrophic factor secretions may be determined as described below.

The protein of the present invention is added to the culture of cells such as human glioblastoma line T98G. At this time, the protein is encapsulated into liposome or bound to lipid in order to enhance the

permeability across the cell membrane. Alternatively, the endoplasmic reticulum-retained sequence, Lys-Asp-Glu-Leu (KDEL), is attached to the C-terminus of the protein in order to make it possible that the protein migrated to the cytoplasm be transported into the ER. The protein thus treated is added to the culture, and the culture is incubated for several days, so as to give culture supernatant. Facilitatory effect of the resultant culture supernatant on secretion of neurotrophic factor is estimated in accordance with a similar procedure to the above manner wherein the facilitatory effect of DNA is estimated.

Compositions for facilitating neurotrophic factor secretions according to the invention may comprise a substance for enhancing the expression level of WAR-1 gene or a substance for enhancing the production level of WAR-1 protein. Determination of facilitatory effect of such substance on neurotrophic factor secretions may be conducted by the following methods.

Selection for a substance for enhancing the expression level of WAR-1 gene or a substance for enhancing the production level of WAR-1 protein may be conducted directly by examining the accumulation of WAR-1 protein within cells using antibodies directed to WAR-1 protein. The detection may be conducted by attaching a tag sequence to WAR-1 protein, and using an anti-Tag antibody.

Additionally, the detection may be conducted by examining the accumulation of certain secretory protein or cell membrane protein factors related to WAR-1 in the ER. When using secretions of certain secretory protein factors as indicator, ELISA, etc., may be used to

examine the accumulation of the proteins in culture supernatant of cells. Further, When using accumulation of certain cell membrane protein factors at cell membrane as indicator, the accumulation may be examined by Western blotting or FACS analysis using antibodies against these factors, or on the basis of increased binding capability to certain ligands if these factors are receptors for the ligands.

Estimation for effects on cells is also applicable. For example, biological effects such as a neurite extension effect of factors that are released from certain cells affected with the above substance and that are accumulated in culture supernatant may be also used in the estimation as described herein.

Alternatively, determination whether or not gene expression level is enhanced may be conducted directly by examining enhanced expression of WAR-1 gene via Northern blotting or RT-PCR. Further, effects of the substances may be also examined by use of activity of reporter gene as indicator by introducing into certain cells expression vectors wherein 5'-upstream sequence containing the promoter region of WAR-1 gene is attached to reporter genes such as lacZ, the luciferase gene, and GFP.

DNAs or proteins of the present invention may be used as active ingredients in pharmaceutical compositions for treating neurodegenerative disorders since they have facilitatory effects on neurotrophic factor secretions. Neurodegenerative disorders are diseases associated with neuro-degeneration or deficiency that are affected by complex actions of an oxidative stress, a neurotoxin, or a genetic factor, and specifically include Alzheimer's disease, cerebral

infarction dementia, Parkinson's disease, Huntington disease, and ALS.

Pharmaceutical compositions for treating neurodegenerative disorders according to the present invention may be used as (1) pharmaceutical compositions comprising WAR-1 protein itself or a portion thereof as an active ingredient, (2) may be used as compositions for gene therapy that lead to expression in body of the DNA encoding WAR-1 that is administered to patients as an active ingredient, or (3) may comprise as an active ingredient a substance for enhancing the expression level of WAR-1 gene or the production level of WAR-1 polypeptide.

Target cells of the pharmaceutical compositions for treating neurodegenerative disorders according to the present invention may be either glial cells or nerve cells, although neurotrophic factors responsible for neuronal survival and maintenance is released from glial cells to act on nerve cells in a paracrine fashion, or is released from nerve cells to act in an autocrine fashion.

(1) Pharmaceutical compositions for treating neurodegenerative disorders which comprise WAR-1 protein itself or a portion thereof as an active ingredient:

The pharmaceutical compositions which comprise WAR-1 protein itself or a portion thereof as an active ingredient may be administered in a similar manner to the composition for inhibiting cancer cell proliferation comprising WAR-1 protein as an active ingredient as described above; and

(2) Pharmaceutical compositions for treating neurodegenerative



disorders which comprise the DNA encoding WAR-1 as an active ingredient:

When the compositions are used as compositions for gene therapy, WAR-1 polypeptide may be produced in a large amount within glial cells or nerve cells resided in neurodegenerative tissues, and thus releases of neurotrophic factor may be facilitated.

When the composition for gene therapy comprising the DNA of WAR-1 is administered, the dosage form may be classified into two types, namely, the manner wherein non-viral vectors are used and the manner wherein viral vectors are used. The details are similar to those of the case wherein the compositions for inhibiting proliferation of cancer cells are used.

Among the viral vectors, adenovirus vector system is most preferably used in the pharmaceutical compositions for treating neurodegenerative disorders since adenovirus is known to be extremely higher than other viral vectors in terms of infection efficiency, and to express foreign genes in even nondividing cells such as nerve cells.

Sendai virus vector system recognized to infect to nerve cells, and herpesvirus vector system when targeting nerve cells are preferred ones next to adenovirus vector system.

Although the amount of the DNA in the formulations may vary appropriately depending on the disease to be treated, the age and weight of the patient, and the like, it is typical to administer 0.0001-100 mg, preferably 0.001-10 mg, of a DNA of the present invention every several days to every several months.

(3) Pharmaceutical compositions for treating neurodegenerative

disorders which comprise as an active ingredient a substance for enhancing the expression level of WAR-1 gene or the production level of WAR-1 polypeptide:

Not only pharmaceutical compositions for treating neurodegenerative disorders which comprise the DNA or the protein of the present invention as an active ingredient, but also the compositions which comprise as an active ingredient a factor or a compound inducing the expression of WAR-1 gene in living bodies or the production of the protein are found to provide facilitatory effects on neurotrophic factor secretions. Accordingly, pharmaceutical compositions for treating neurodegenerative disorders which comprise as an active ingredient such substances as those facilitating the WAR-1 gene expression, or such substances as those facilitating the WAR-1 protein production are fallen within the scope of the present invention. Such substances include peptides, analogues thereof, microorganism cultures, synthesized compounds, and the like.

#### BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the homology comparisons among the base sequences of cDNAs encoding TRAM from human (HTRAM in the figure), KIAA0057 from human (KIAA0057 in the figure), WAR-1 from human (HWAR1 in the figure), and WAR-1 from rat (RWAR1 in the figure).

Figure 2 shows the homology comparisons among the amino acid sequences deduced from the base sequences of cDNAs encoding TRAM from human (HTRAM in the figure), KIAA0057 from human (KIAA0057 in the figure), WAR-1 from human (HWAR1 in the figure),

and WAR-1 from rat (RWAR1 in the figure).

Figure 3 shows the photograph of the electrophoretic profiles analyzed by RT-PCR of the expression of hWAR-1 gene in various cancer cell lines from human. Upper lane shows the RT-PCR result of hWAR-1, whereas the lower lane shows the RT-PCR result of hTRAM.

Figure 4 shows the photograph of the electrophoretic profiles analyzed by Northern hybridization of the expression of hWAR-1 gene in various human tissues. Numbers indicated at the right side show size markers of RNA molecular weight (Kb).

Figure 5 shows the photograph of the electrophoretic profiles analyzed by Northern hybridization of the expression of hTRAM gene in various human tissues. Numbers indicated at the right side show size markers of RNA molecular weight (Kb).

Figure 6 shows the photomicrography of the morphologic changes of human glioblastoma, T98G, infected with the adenovirus incorporated with hWAR-1 gene. A: cells infected with no adenovirus, B: adenovirus-infected cells in which only adenovirus vector is infected, C: cells infected with recombinant adenovirus expressing the hWAR-1 sense strand, and D: cells infected with recombinant adenovirus expressing the hWAR-1 antisense strand.

Figure 7 shows the photomicrography of the morphologic change of T98G cells infected with the adenovirus vector, AxCAwt, with the time course. A: immediately after the infection, B: eight hours after the infection, C: 21 hours after the infection (after about one day), D: 31 hours after the infection (after about 1.5 days), E: 46 hours after the infection (after about two days), and F: 70 hours after the infection

(after about three days).

Figure 8 shows the photomicrography of the morphologic changes of T98G cells infected with the recombinant adenovirus vector, AxCAWAR1-L, expressing the sense strand of hWAR-1, with the time course. A: immediately after the infection, B: eight hours after the infection, C: 21 hours after the infection (after about one day), D: 31 hours after the infection (after about 1.5 days), E: 46 hours after the infection (after about two days), and F: 70 hours after the infection (after about three days).

Figure 9 shows the photograph of the electrophoretic profiles analyzed by Northern hybridization using oligonucleotide to detect rWAR-1 gene from rat, which shows the expression of the rWAR-1 gene in various rat tissues. Two bands of the approximately 2.4 Kb transcription products were detected in the tissues wherein the gene was expressed.

Figure 10 shows the photograph of the electrophoretic profiles analyzed by Northern hybridization using, as probe, 1.9Kb EcoRI fragment to detect rWAR-1 gene from rat. Lane 1: juvenile rat hippocampus poly(A) mRNA; Lane 2: adult rat brain poly(A) mRNA; Lane 3: rat retina poly(A) mRNA.

Figure 11 shows the photograph of the electrophoretic profiles analyzed by Northern hybridization of the poly(A) mRNA prepared from various sites of human brain using as probe the DNA fragment detecting specifically hWAR-1 gene from human. Numbers indicated at the right side show size markers of RNA molecular weight (Kb).

## EXAMPLES

The present invention is further illustrated by the following examples, but is not restricted by these examples in any way.

## 5 Example 1

Preparation of rat cDNA library

In order to clone various new cDNAs, cDNA library was prepared from juvenile rat as shown below.

10 First of all, tissues had been removed from juvenile rats aged 12 days after birth were homogenized before adding guanidine thiocyanate, and then cesium chloride density gradient centrifugation was performed on the homogenate to prepare 2 mg of total RNA in usual manners.

15 The total RNA was applied to oligo(dT) cellulose column (Pharmacia) to purify 103  $\mu$ g of mRNA having a poly(A) supplemental sequence. Single-stranded complementary DNA was synthesized with reverse transcriptase using oligo(dT) primer and random primers, based on 16  $\mu$ g aliquot of the mRNA. Then, RNaseH and E.coli DNA polymerase I was reacted with the DNA to synthesize double-stranded  
20 cDNA. The double-stranded cDNA was blunt-ended by treating it with T4 DNA polymerase, and EcoRI-adaptors were attached to the cDNA at both terminuses. Final amounts of the cDNA when using the oligo(dT) primer and the random primers were 2  $\mu$ g and 1.3  $\mu$ g, respectively. A  
25  $\lambda$  phage vector,  $\lambda$ gt10, that had been incorporated with the aliquot of the cDNA at the EcoRI cleavage site was introduced into  $\lambda$  phage particles with an *in vitro* packaging kit (Stratagene), and infected to *E.*

*coli* C600<sup>h<sup>n</sup></sup> (Stratagene) to prepare the intended cDNA library of juvenile rat. Plaque forming units (pfu) of the cDNA synthesized with oligo(dT) primer and random primers were  $8.8 \times 10^7$  pfu and  $2.5 \times 10^7$  pfu per  $\mu$ g, respectively.

## Example 2

### Determination of the base sequence of cDNA encoding rWAR-1

$\lambda$  phage DNAs were recovered from clones randomly selected from the cDNA library prepared in Example 1 by the usual plate lysine method, and cleaved with restriction enzyme EcoRI, followed by subcloning the inserted cDNA portions into M13 phage vector. Base sequence of the inserted cDNA portion in one of them was determined, revealing that the cloned cDNA is approximately 2.2 Kb in length, and has an open reading frame spanning approximately 1 Kb. This was predicted to be a full length cDNA in consideration of the poly(A) sequence length since the mRNA corresponding to the cDNA was estimated approximately 2.4 Kb in size according to Northern analysis using the 1.8 Kb EcoRI fragment as probe in usual manners. The resultant clone was named clone 12. Search of the base sequence of cDNA of clone 12 on GenBank databases revealed that the cDNA of clone 12 is novel since no sequence that has the identical sequences was searched, although it shares 59.7% homology in terms of base sequence and 57.0% homology in terms of amino acid sequence with TRAM that is an ER membrane transport-associated protein from human (GenBank Accession No. X63679), whereas it shares 53.7% homology in terms of base sequence and 41.0% homology in terms of

amino acid sequence with KIAA0057 (GenBank Accession No. D31762) (Table 1). The protein encoded by the new cDNA was named rat WAR-1 (rWAR-1). Base sequence and deduced amino acid sequence of rWAR-1 are shown in SEQ ID: Nos. 1 and 2. Additionally, the base sequence and the amino acid sequence of known human TRAM (hTRAM) are shown in SEQ ID: Nos. 5 and 6, and the base sequence and the amino acid sequence of human KIAA0057 are shown in SEQ ID: Nos. 12 and 13.

Table 1

% Homology between human WAR-1, human TRAM, rat WAR-1 and KIAA0057

		Human WAR-1	Rat WAR-1	Human TRAM	KIAA 0057
Human WAR-1	base sequence (amino acid sequence)		72.4 (72.7)	76.3 (70.7)	64.1 (44.1)
Rat WAR-1	base sequence (amino acid sequence)	72.4 (72.7)		59.7 (57.0)	53.7 (41.0)
Human TRAM	base sequence (amino acid sequence)	76.3 (70.7)	59.7 (57.0)		69.3 (51.3)
KIAA 0057	base sequence (amino acid sequence)	64.1 (44.1)	53.7 (41.0)	69.3 (51.3)	

*E. coli* DH5 $\alpha$  (prWAR-1) that contains prWAR-1 wherein the cDNA fragment of rWAR-1 as described above is incorporated into a vector pBlueScript II was deposited at The National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, 1-1-3 Higashi, Tsukuba, Ibaraki, Japan (description of

microorganism: *E. coli* DH5 $\alpha$  (prWAR-1); deposition date: October 6, 1998; deposit number: FERM P-17018).

### Example 3

5     Cloning of cDNA encoding hWAR-1 and determination of its base sequence

10     Clone having the cDNA of human type WAR-1 was screened from human cDNA library (Clontech) in usual manners using as probe the 0.8 Kb EcoRI-XhoI DNA fragment corresponding to the open reading frame of rWAR-1. As a result, one clone was isolated from the human cDNA library at  $1 \times 10^6$  pfu. Determination of the base sequence revealed that the base sequence shares 72.4% homology and the amino acid sequence shares 72.7% homology with those of raWAR-1 as obtained in Example 2, concluding that this should be cDNA encoding human type WAR-1 (hWAR-1) (Table 1). The determined base sequence and the deduced amino acid sequence are shown in SEQ ID: Nos. 3 and 4. The hWAR-1 shares 76.3% homology in terms of base sequence and 70.7% homology in terms of amino acid sequence with TRAM as mentioned above, whereas it shares 64.1% homology in terms of base sequence and 44.1% homology in terms of amino acid sequence with KIAA0057 (Table 1).

25     *E. coli* DH5 $\alpha$  (phWAR-1) that contains phWAR-1 wherein the cDNA fragment of hWAR-1 as described above is incorporated into a vector pBluescript II was deposited at The National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, 1-1-3 Higashi, Tsukuba, Ibaraki, Japan (description of



microorganism: *E. coli* DH5 $\alpha$  (phWAR-1); deposition date: October 6, 1998; deposit number: FERM P-17019).

Comparison between the base sequences of cDNAs encoding hWAR-1, rWAR-1, hTRAM and KIAA0057 is shown in Figure 1.

5 Comparison between the amino acid sequences of hWAR-1, rWAR-1, hTRAM and KIAA0057 is shown in Figure 2.

#### Example 4

##### Examination of expression of the gene encoding hWAR-1 in various human cancer established cell lines

10 For the purpose of assessment of expression of the genes encoding hWAR-1 and hTRAM in different types of human cancer cell, primers for each of specific amplifications of hWAR-1 and hTRAM mRNAs were designed to subject to RT-PCR under the following conditions.

15 For hWAR-1 mRNA amplification, the segments corresponding to positions 823-846 (SEQ ID NO:7) and the positions 1093-1116 (SEQ ID NO:8) of the hWAR-1 base sequence depicted in Figure 1 were used as 5' and 3' primers, respectively. For hTRAM mRNA amplification, 20 the segments corresponding to positions 823-846 (SEQ ID NO:9) and positions 1087-1110 (SEQ ID NO:10) of the hTRAM base sequence depicted in Figure 1 were used as 5' and 3' primers, respectively.

25 As human cancer cell lines, uterocervical cancer cell line Hela, lung cancer cell line A549, bladder carcinoma cell line T24, large bowel cancer cell line SW480, glioblastoma cell line T98G, hepatoma cell line HepG2, Wilms' tumor: renal carcinoma cell line G401, Burkitt

lymphoma: B lymphoma cell line Daudi, and T lymphoma cell line MOLT4, Jurkat were used. These cell lines are all available from DAINIPPON PHARMACEUTICAL CO., LTD., except for T24 cell line that is available from RIKEN GENE BANK.

5 RT-PCR reaction was conducted as follows.

10 Firstly, total RNA was prepared from each cell line described above using a total RNA purification kit (NIPPON GENE CO., LTD.). To 4 µg (9.5 µl) of the total RNA, 2.0 µl of 100mM DTT, 4.0 µl of 5 x First Standard Buffer (Gibco BRL), 0.5 µl of 40 U/µl RNasein (Promega), 2.0 µl of 10mM dNTP, 1.0 µl of 0.2 µg/µl pd(N)<sub>6</sub> primer, 1.0 µl of 200 U/µl MMTV-RT (Gibco BRL) were added, and the mixture was warmed to 37°C for 45 minutes, followed by heated to 95°C for 5 minutes to inactivate the enzymes. Then, a 16 µl aliquot was removed from the reaction mixture and added with 24 µl of H<sub>2</sub>O. To 2.0 µl of this mixture, 2.5 µl of 10XPCR Buffer (Perkin Elmer), 2.5 µl of 2.0mM dNTP, 25 µM of each 5' primer, 25 µM of each 3' primer, 16.875 µl of H<sub>2</sub>O and 0.125 µl of 5 U/µl AmpliTaq Gold (Perkin Elmer) were added. The mixture was heated to 95°C for 9 minutes, and was subjected to 35 cycles of the reactions at 95°C for 1 minute, 60°C for 1 minute and 72°C for 2 minutes. Finally, the mixture was heated to 72°C for 10 minutes, and then 3 µl of this mixture was subjected to 2% agarose gel electrophoresis. The results are shown in Figure 3.

25 Expression of hTRAM was observed in all of these cancer cell lines, and that expression of hWAR-1 was shown to be somewhat lower in Jurkat etc. than that of hTRAM, indicating that hWAR-1 gene differs from hTRAM gene (Figure 3) in terms of expression manner.

## Example 5

Examination of expression in normal human tissue of the gene encoding hWAR-1

5 In order to examine expressions of hWAR-1 and hTRAM genes in tissues, the following experiments were conducted.

10 First, the hWAR-1 gene fragment of interest was amplified by means of RT-PCR as described in Example 4 using the above primer sequence specific for hWAR-1 (SEQ ID NOs:7 and 8), and the amplified fragments were cloned to pT7Blue(R)T vector (Novagen). Like this, the hTRAM gene fragment of interest was amplified by means of RT-PCR using the primer sequence specific for hTRAM (SEQ ID NOs:9 and 10) and cloned to the vector. RT-PCR was conducted using these plasmids as template and the primer sequences (SEQ ID NOs:9 and 10), and the amplified DNA fragments were then collected by polyacrylamide gel electrophoresis.

15 Subsequently, resultant DNA fragments were labeled with <sup>32</sup>P according to multiprime labeling method to prepare probes, and were hybridized onto MTN blot filter purchased from CLONTECH Laboratories, Ltd. (human MTN blot I and human MTN blot II) according to conventional techniques.  $\beta$ -actin (CLONTECH Laboratories, Ltd.) was used as a control probe. The results of hWAR-1 and TRAM gene expressions in tissues are shown in Figures 4 and 5, respectively. Expression of hTRAM was observed in all of the tissues (Figure 5), whereas expression of hWAR-1 was not observed in tissues such as lymphatic system (spleen, thymus and lymphocyte), lung and

20

25

liver (Figure 4). Based on the results of Example 4 showing that expression of hTRAM gene was observed in the cell lines from lung cancer (A459), T lymphoma (MOLT4, Jurkat) and hepatoma (HepG2) (Figure 3), expression of hTRAM in these tissues was shown to develop in association with malignant transformation.

Intensity of the bands obtained in the autoradiogram was analyzed to examine the expression level in each organ, comparing with the intensity of  $\beta$ -actin as standard. The results of this examination are shown in Table 2. Values shown in the table has been standardized for the expression in testis as 1.00.

Table 2

Tissue	hWAR-1/ $\beta$ -actin	Tissue	hWAR-1/ $\beta$ -actin
Heart	0.56	Spleen	0.00
Brain	1.47	Thymus	0.00
Placenta	0.00	Prostate	0.28
Lung	0.00	Testis	1.00
Liver	0.00	Ovary	0.25
Skeletal muscle	0.00	Small intestine	0.15
Kidney	0.74	Colon	0.12
Pancreas	1.21	Peripheral leukocyte	0.00

Table 2 apparently shows that hWAR-1 gene was expressed most highly in brain, similar to rWAR-1 gene, and was expressed more highly in pancreas, kidney and testis.

### Example 6

#### Construction of recombinant cosmid vector

Cosmid vectors incorporated with the sense or antisense strand of hWAR-1 were constructed using the approximately 1.3 Kb DNA

fragment of the cDNA encoding hWAR-1 spanning from the *PvuII* cleavage site located 36 base pairs upstream of the ATG initiation codon to *DraI* cleavage site located 139 base pairs downstream of the TAA termination codon. Specifically, said 1.3 Kb *PvuII*/*DraI* fragment of hWAR-1 was ligated to a *SwaI*-cleaved pAxCawt cosmid vector (described in Kanegae et al., 1995, Nucleic Acid Res., 23, 3816-3821 and available as adenovirus expression vector kit form TAKARA SHUZO CO., LTD.). Then, any cosmid vector not containing the insertion sequence was digested with *SwaI*, and an aliquot of the reaction mixture was *in vitro* packaged according to the conventional techniques. From the developed colonies obtained after infection of *E. coli* DH5 $\alpha$ , cosmid DNAs were recovered, cleaved with *EcoRI*/*XhoI* and the DNA was analyzed on 1% agarose gel electrophoresis. As a result, nine 9 clones expressing the sense strand RNA and six clones expressing the antisense strand RNA were obtained, and, among them, each three clones were selected. Those expressing the sense strand RNA were cleaved with *EcoRI*/*XbaI* or *BglII*, and those expressing the antisense strand RNA were cleaved with *StuI*/*XbaI* or *EcoRI*/*XhoI* to check the direction and the integrity of the inserted DNA fragment.

## Example 7

### Preparation of recombinant adenovirus

A recombinant adenovirus vector wherein the expression unit of hWAR-1 gene (sense/antisense) as constructed in Example 6 was inserted into E1 gene deletion site of a non-proliferative recombinant adenovirus vector derived from human adenovirus type 5 (E1 and E3

genes have been deleted), was prepared according to the following procedures. As promoter, the CAG promoter was used, which is disclosed as a high expression vector in Japanese Patent Publication (kokai) No. 3-168087, and the preparation of the recombinant  
5 adenovirus was conducted according to the ordinary techniques (Miyake et al., Proc. Natl. Acad. Sci., Vol.93, 1320-1324(1996) and Japanese Patent Publication No. 7-298877).

A virus DNA-terminal protein complex was prepared according to the ordinary technique (Japanese Patent Publication No. 7-298877) from the recombinant adenovirus vector AxCAwt (described in Kanegae  
10 et al., 1995, Nucleic Acid Res., 23, 3816-3821 and available as adenovirus expression vector kit form TAKARA SHUZO CO., LTD.) wherein only CAG promoter was inserted into the E1 gene deletion site of the adenovirus, and the complex was digested simultaneously with the restriction enzymes EcoT221 and ClaI. The restriction enzyme-  
15 digested virus DNA-terminal protein complex and the cosmid vector inserted with the hWAR-1 sense or antisense strand as prepared in Example 6 were used to transform 293 cells by means of calcium phosphate co-precipitation. After cloning the resultant recombinant  
20 adenovirus, the virus DNAs were digested with *Xho*I and *Cla*I to screen the virus of interest, thus obtaining adenovirus vectors AxCAWAR1-L (sense strand) and AxCAWAR1-R (antisense strand). These virus were passaged four times to obtain the virus solution, and the titer of the  
25 virus solution was then determined according to a ordinary technique as described in Japanese Patent Publication (kokai) No. 7-298877. In the subsequent experiments, the virus solution was used.

## Example 8

Expression of hWAR-1 gene by infection of recombinant adenovirus vector

Human glioblastoma cell line T98G was infected at 10 multiplicity of infection with the adenovirus vectors AxCAWAR1-L or AxCAWAR1-R, and AxCAwt (control virus) as prepared in Example 7 at 37°C for 1 hour, and then cultured in a minimum nutrient culture medium containing 5% FCS. On the next day of the infection, the culture medium was replaced with a reduced serum medium (0.5% FCS). On the second day after the infection, all of the cells infected with adenoviruses degenerated in comparison with the cells subjected to infection procedure in culture medium alone, showing that the adenovirus infection affected somewhat. However, remarkable change in cell morphology was observed in the cells infected with AxCAWAR1-L expressing the sense strand (Figure 6). No difference in cell morphology was observed between the cells infected with AxCAwt and the cells infected with AxCAWAR1-R. T98G cells infected with AxCAWAR1-L underwent cell death three days after the infection.

## Example 9

Morphologic change of T98G cell by WAR-1 sense RNA expression

T98G cells were adjusted to a low density (about 1/10 of the confluent density in Example 8) so that morphologic change of the cells could be easily observed, then were infected as described in Example 8 with the adenovirus vector AxCAWAR1-L (sense strand) or AxCAWAR1-

R (antisense strand), and AxCAwt (control), and were observed for their morphologic change with time course. On the next day of the infection, even the control cells infected with AxCAwt underwent degeneration relatively to the cells subjected to infection procedure in culture medium alone, showing that the adenovirus infection affected somewhat was found (Figure 7). However, in comparison with the cells infected with AxCAwt, the cells infected with AxCAWAR1-L expressing the hWAR-1 sense strand showed remarkable morphologic change on the next day of the infection (21-31 hours later of the infection), and underwent cell death on the third day after the infection (Figure 8). No morphologic difference between the cells infected with AxCAWAR1-R expressing antisense RNA and the cells infected with AxCAwt was found.

#### Example 10

##### Morphologic change of T98G cell by addition of WAR-1 protein

WAR-1 protein is previously enclosed into liposome or bound to lipid to enhance its permeability across cell membrane. Endoplasmic reticulum-retained sequence, Lys-Asp-Glu-Leu (KDEL), is attached to the C-terminus of the protein so that the protein incorporated into cytoplasm can be transported to endoplasmic reticulum. The protein of the invention thus treated is added to a culture solution of T98G as used in Examples 8 and 9, and the culture solution is cultured for several days. Thereafter, the cell counts of T98G cells and the morphologic change of the cell can be observed to determine inhibitory effect on cancer cell proliferation. Also, inhibitory effect on cancer cell



proliferation can be determined by measuring the decrease in its ability to incorporate  $^3\text{H}$ -labeled thymidine (Nagase et al., Int. J. Cancer, 65, 620-626, 1996).

5      Example 11

Examination of the expression of rat WAR-1 gene in tissues

Hybridization using an oligonucleotide probe which specifically detect rWAR-1 gene was performed onto rat MTN blot filter purchased from CLONTECH. The nucleotide sequence of the probe used in the hybridization was

10      ATTTTCTGTGCCTTTTCTCGACCTGGACCGTCTCTTCCTCCCACAGACA  
as described in SEQ ID NO:11. Hybridization conditions were in accordance with the protocol of CLONTECH. As a result of the hybridization, rWAR-1 gene was expressed intensively in brain, which are as shown in Figure 9. Also, the expression of this gene was  
15      observed in testis, whereas the expressions in lung and kidney were weak.

Example 12

20      Examination of human and rat WAR-1 expressions in nerve related sites

Northern hybridizations were conducted using rWAR-1 gene as a probe with a poly(A)mRNA prepared from juvenile rat hippocampus according to a ordinary method, and with mature rat brain and retina poly(A)mRNAs purchased from CLONTECH. The result shows that  
25      expression of rWAR-1 gene was also observed in retina as shown in

Figure 10. Decreased intensity in the detected bands were believed to be caused by degradation of poly(A)mRNA.

Further, using a probe for detecting hWAR-1 gene as used in Example 5, hybridization was conducted onto MTN blot filter purchased from CLONTECH (human brain MTN blot II and III) (Figure 1). The result shows that expression of hWAR-1 gene was observed in all sites in brain. Expression of the gene was also observed in spinal cord. It suggested that hWAR-1 gene also expressed in peripheral nervous system.

### Example 13

#### Assessment of neurite extension effect on PC12 cell using the culture supernatants of the cells infected with adenovirus vectors

Human glioblastoma cell line T98G was infected at 10 multiplicity of infection with the adenovirus vectors AxCAWAR1-L or AxCAWAR1-R, or AxCAwt (control virus) at 37°C for 1 hour and cultured in a minimum nutrient culture medium containing 5% FCS. Same virus infection study was conducted for human lung cancer A549 cell, which is a non-nervous system culture, as a negative control. Supernatant from each culture after incubation for two days was collected and centrifuged, and the culture supernatant was then passed through a 0.22-micron membrane filter to remove cell debris.

Optionally, obtained culture supernatant was fivefold concentrated using Centriprep (3000Kd cut)(Amicon Corporation).

PC12 cells in 100  $\mu$ l of DMEM-10%FCS medium was plated on a collagen-coated 96-well plate at  $1-5 \times 10^4$  cells/ml per well, and the

plate was incubated for four days. The culture supernatant was discarded, and the supernatants obtained from the cell culture infected with recombinant adenovirus were added up to 100  $\mu$ l of final volume as shown in Tables 3 and 4. Final serum concentration of the culture was adjusted to 10% FCS. As a sample to be added, culture medium, the culture supernatant of the non-infected cell (Mock), the culture supernatant of the cell infected with AxCAWAR1-L (WAR(+)), and the culture supernatant of the cell infected with AxCAwt (control) were used. 10  $\mu$ l of 500ng/ml  $\beta$ -NGF was added as a positive control in this assay system.

After addition of a culture supernatant, incubation was continued for two days, and neurite extension effect on PC12 cells was observed microscopically. Potent neurite extension effect was observed only in PC12 cells added with the culture supernatant of T98G cells infected with the adenovirus expressing hWAR-1 sense strand. Neurite extension effect was not found in all experiments using the culture supernatant of A549 cells. The results of Table 4 confirmed that at least 50  $\mu$ l of the culture supernatant of the cell infected with AxCAWAR1-L was enough to raise neuritic outgrowth effect.

The above results distinctly show that the secretion to extracellular space of the neurotrophic factor produced in T98G cells was enhanced by the expression of WAR-1 gene. The Table 3 shows the results of PC12 assay using the culture supernatants of T98G and A549 cells infected with adenovirus (100  $\mu$ l of the supernatant from each infected cell was added). In Table 3, "+++", "++", "+" and "-" shows the neurite extension of PC12 cells in order of its magnitude.

Table 3

	$\beta$ -NGF	T98G	A549
Medium	—	—	—
Mock		+	—
Control		+	+
WAR(-)		+	+
WAR(+)		++	+
$\beta$ -NGF	+++		

Table 4 shows the results of PC12 assay using the culture supernatants of T98G cells infected with adenovirus. In Table 4, “+++”, “++”, “+”, “-/+” and “-” shows the neurite extension of PC12 cells in order of its magnitude.

Table 4

	$\beta$ -NGF	condensed culture supernatant ( $\mu$ l)			culture supernatant ( $\mu$ l)		
		50	30	20	100	50	30
Medium	—	—	—	—	—	—	—
Mock		-/+	-/+	-/+	-/+	-/+	-/+
Control		++	+	+	+	+	+
WAR(-)		-/+	+	+	-/+	++	-/+
WAR(+)		++	++	++	++	++	+
$\beta$ -NGF	+++						

## INDUSTRIAL APPLICABILITY

DNAs of the present invention encode WAR-1 that has a

inhibitory effect on cancer cell proliferations, and production or induction therefor of WAR-1 polypeptide encoded by the DNA within cells makes possible inhibition of cancer cell proliferation, which leads to cell death of cancer cells. The invention provides a new therapy for cancers based on such actions of WAR-1.

Additionally, the production or induction therefor makes possible prevention from neuro-deficiency at neurodegenerative areas in central and peripheral nervous systems, and at the same time facilitation of secretions of diverse nerve growth factors useful for activation of cells just before entering into cell death and recovering to normal neurotransmission. The invention provides a new therapy for neurodegenerative diseases.

## C L A I M S

1. A DNA encoding a protein selected from a group consisting of:

- 5 (a) a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, and
- (b) a protein comprising an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein
- 10 having an inhibitory effect on cancer cell proliferations.

2. A DNA selected from a group consisting of

- (c) a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, and
- (d) a DNA that hybridizes with the DNA of the above (c) under stringent
- 15 conditions, and encodes a protein having an inhibitory effect on cancer cell proliferations.

3. The DNA of claim 2, which is cloned from chromosomal DNA libraries using all or part of the DNA of SEQ ID: No. 1 or SEQ ID: No. 3 as probe.

4. The DNA of claim 3, which contains a promoter region.

5. The DNA of claim 1 or 2, which is contained in the microorganism of deposit number FERM BP-6910 or FERM BP-6911.

6. A protein obtainable by the expression of the DNA of any one of claims 1 to 5.

7. A recombinant expression vector comprising the DNA of any one of claims 1 to 5.

5

10

15

20

25

16. A pharmaceutical composition comprising the DNA of any

one of claims 1 to 5, or the protein of claim 6 as an active ingredient.

17. A composition for inhibiting proliferation of cancer cells, which is characterized in that the composition enhances the expression level of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3.

18. A composition for inhibiting proliferation of cancer cells, which comprises the DNA of any one of claims 1 to 5 as an active ingredient.

19. A composition for inhibiting proliferation of cancer cells of claim 18, which comprises an adenovirus vector.

20. A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a DNA encoding a protein selected from a group consisting of:

(a) a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, and

(b) a protein comprising an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein having a facilitatory effect on neurotrophic factor secretions.

21. A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a DNA selected from a group consisting of:

(c) a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, and

(d) a DNA that hybridizes with the DNA of the above (c) under stringent conditions, and encodes a protein having a facilitatory effect on



neurotrophic factor secretions.

22. The composition for facilitating neurotrophic factor secretions of claim 21, which comprises as an active ingredient a DNA that is cloned from chromosomal DNA libraries using all or part of the DNA of SEQ ID: No. 1 or SEQ ID: No. 3 as probe.

23. The composition for facilitating neurotrophic factor secretions of claim 22, which comprises as an active ingredient the DNA that contains a promoter region.

24. The composition for facilitating neurotrophic factor secretions of claim 20 or 21, which comprises as an active ingredient a DNA that is contained in the microorganism of deposit number FERM BP-6910, or FERM BP-6911.

25. The composition for facilitating neurotrophic factor secretions of any one of claims 20 to 24, wherein the DNA is comprised in a recombinant expression vector.

26. The composition for facilitating neurotrophic factor secretions of claim 25, wherein the DNA is comprised in an adenovirus vector.

27. A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a protein selected from a group consisting of:

(a) a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, and

(b) ~~a protein comprising~~ an amino acid sequence containing deletion, substitution and/or addition of one or more amino acid residues in the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4, said protein

having a facilitatory effect on neurotrophic factor secretions.

28. A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a protein encoded by a DNA selected from a group consisting of:

5 (c) a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, and

(d) a DNA that hybridizes with the DNA of the above(c) under stringent conditions, and encodes a protein having a facilitatory effect on neurotrophic factor secretions.

10 29. The composition for facilitating neurotrophic factor secretions of claim 27 or 28, which comprises as an active ingredient a protein encoded by a DNA that is contained in the microorganism of deposit number FERM BP-6910, or FERM BP-6911.

15 30. A composition for facilitating neurotrophic factor secretions, which comprises as an active ingredient a substance for enhancing the expression level of the DNA of SEQ ID: No. 1 or SEQ ID: No. 3 or a substance for enhancing the production level of a protein comprising the amino acid sequence of SEQ ID: No. 2 or SEQ ID: No. 4.

20 31. A pharmaceutical composition for treating neurodegenerative diseases, which comprises the composition for facilitating neurotrophic factor secretions of any one of claims 20 to 30.

25 32. A method for facilitating secretion of neurotrophic factors, which comprises enhancing the expression level of a DNA comprising the base sequence of SEQ ID: No. 1 or SEQ ID: No. 3, or enhancing the

production level of a protein comprising the amino acid sequence of  
SEQ ID: No. 2 or SEQ ID: No. 4.

09807470.050401

PLEASE NOTE:  
YOU MUST  
COMPLETE THE  
FOLLOWING

# BIRCH, STEWART, KOLASCH & BIRCH, LLP

P.O. Box 747 • Falls Church, Virginia 22040-0747  
Telephone: (703) 205-8000 • Facsimile: (703) 205-8050

Attorney Docket No.  
0020-4850P

## COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL PROTEIN WAR-1 AND GENE THEREOF

Insert Title:

Fill in Appropriate  
Information -  
For Use Without  
Specification  
Attached:

the specification of which is attached hereto. If not attached hereto,  
the specification was filed on April 13, 2001 as  
United States Application Number \_\_\_\_\_;  
and amended on \_\_\_\_\_ (if applicable) and/or  
the specification was filed on October 13, 1999 as PCT  
International Application Number PCT/JP99/05631; and was  
amended under PCT Article 19 on \_\_\_\_\_ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

(Number)	(Country)	(Month/Day/Year Filed)	Priority Claimed
<u>290711/1998</u>	<u>Japan</u>	<u>October 13, 1998</u>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below.

Insert Provisional  
Application(s):  
(if any)

(Application Number)	(Filing Date)
_____	_____
_____	_____

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Country	Application Number	Date of Filing (Month/Day/Year)
_____	_____	_____
_____	_____	_____

Insert Requested  
Information:  
(if appropriate)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Insert Prior U.S.  
Application(s):  
(if any)

(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)
_____	_____	_____
_____	_____	_____

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

Raymond C. Stewart (Reg. No. 21,066)  
 Joseph A. Kolasch (Reg. No. 22,463)  
 Bernard L. Sweeney (Reg. No. 24,448)  
 Charles Gorenstein (Reg. No. 29,271)  
 Leonard R. Svensson (Reg. No. 30,330)  
 Andrew D. Meikle (Reg. No. 32,868)  
 Joe McKinney Muncy (Reg. No. 32,334)  
 John W. Bailey (Reg. No. 32,881)  
 Gary D. Yacura (Reg. No. 35,416)

Terrell C. Birch (Reg. No. 19,382)  
 James M. Slattery (Reg. No. 28,380)  
 Michael K. Mutter (Reg. No. 29,680)  
 Gerald M. Murphy, Jr. (Reg. No. 28,977)  
 Terry L. Clark (Reg. No. 32,644)  
 Marc S. Weiner (Reg. No. 32,181)  
 Donald J. Daley (Reg. No. 34,313)  
 John A. Castellano (Reg. No. 35,094)

Send Correspondence to:

**BIRCH, STEWART, KOLASCH & BIRCH, LLP**  
 P.O. Box 747 • Falls Church, Virginia 22040-0747  
 Telephone: (703) 205-8000 • Facsimile: (703) 205-8050

or Customer No. 2292

PLEASE NOTE:  
 YOU MUST  
 COMPLETE  
 THE  
 FOLLOWING:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First  
 or Sole Inventor:  
 Insert Name of  
 Inventor  
 Insert Date This  
 Document is Signed

Insert Residence  
 Insert Citizenship

Insert Post Office  
 Address

Full Name of Second  
 Inventor, if any:  
 see above

Full Name of Third  
 Inventor, if any:  
 see above

Full Name of Fourth  
 Inventor, if any:  
 see above

GIVEN NAME/FAMILY NAME <u>Naoki TOHDOH</u>		INVENTOR'S SIGNATURE <u>Naoki Tohdoh</u>	DATE* April 25, 2001
Residence (City, State & Country) <u>Kobe-shi, Hyogo-ken, Japan JPX</u>		CITIZENSHIP Japan	
MAILING ADDRESS (Complete Street Address including City, State & Country) <u>1612-9, Aza-Hirano, Mikage, Mikage-cho, Higashinada-ku, Kobe-shi, Hyogo-ken, Japan</u>			
GIVEN NAME/FAMILY NAME <u>Tadahiko YOSHIMA</u>		INVENTOR'S SIGNATURE <u>Tadahiko Yoshima</u>	DATE* April 25, 2001
Residence (City, State & Country) <u>Toyono-gun, Osaka-fu, Japan JPX</u>		CITIZENSHIP Japan	
MAILING ADDRESS (Complete Street Address including City, State & Country) <u>4-4-8, Shinkofudai, Toyono-cho, Toyono-gun, Osaka-fu, Japan</u>			
GIVEN NAME/FAMILY NAME <u>Kazuo KOMIYA</u>		INVENTOR'S SIGNATURE <u>Kazuo Komiya</u>	DATE* April 25, 2001
Residence (City, State & Country) <u>Nishinomiya-shi, Hyogo-ken, Japan JPX</u>		CITIZENSHIP Japan	
MAILING ADDRESS (Complete Street Address including City, State & Country) <u>4-15-215, Maruhashi-cho, Nishinomiya-shi, Hyogo-ken, Japan</u>			
GIVEN NAME/FAMILY NAME <u>Shinichiro TOJO</u>		INVENTOR'S SIGNATURE <u>Shinichiro Tojo</u>	DATE* April 25, 2001
Residence (City, State & Country) <u>Ashiya-shi, Hyogo-ken, Japan JPX</u>		CITIZENSHIP Japan	
MAILING ADDRESS (Complete Street Address including City, State & Country) <u>7-26-704, Higashiyama-cho, Ashiya-shi, Hyogo-ken, Japan</u>			

Full Name of Fifth  
Inventor, if any:

5-00 see above

GIVEN NAME/FAMILY NAME <u>Kiyomitsu NEMOTO</u>	INVENTOR'S SIGNATURE <i>K. Nemoto</i>	DATE* April 25, 2001
Residence (City, State & Country) <u>Shizuoka-shi, Shizuoka-ken, Japan JPX</u>		CITIZENSHIP Japan
MAILING ADDRESS (Complete Street Address including City, State & Country) <u>1-8-3-406, Sena, Shizuoka-shi, Shizuoka-ken, Japan</u>		

Full Name of Sixth  
Inventor, if any:

6-00 see above

GIVEN NAME/FAMILY NAME <u>Hironori ISHIKAWA</u>	INVENTOR'S SIGNATURE <i>Hironori Ishikawa</i>	DATE* April 25, 2001
Residence (City, State & Country) <u>Toyonaka-shi, Osaka-fu, Japan JPX</u>		CITIZENSHIP Japan
MAILING ADDRESS (Complete Street Address including City, State & Country) <u>2-10-4-429, Sonehigashi-machi, Toyonaka-shi, Osaka-fu, Japan</u>		

Full Name of Seventh  
Inventor, if any:

7-00 see above

GIVEN NAME/FAMILY NAME <u>Hajime OKUYAMA</u>	INVENTOR'S SIGNATURE <i>Hajime Okuyama</i>	DATE* April 25, 2001
Residence (City, State & Country) <u>Nishinomiya-shi, Hyogo-ken, Japan JPX</u>		CITIZENSHIP Japan
MAILING ADDRESS (Complete Street Address including City, State & Country) <u>4-15-524, Maruhashi-cho, Nishinomiya-shi, Hyogo-ken, Japan</u>		

Full Name of Eighth  
Inventor, if any:

see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Ninth  
Inventor, if any:

see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Tenth  
Inventor, if any:

see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Eleventh  
Inventor, if any:

see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Twelfth  
Inventor, if any:

see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

1/25

## SEQUENCE LISTING

&lt;110&gt; Sumitomo Pharmaceuticals Company, Limited

&lt;120&gt; Novel protein WAR-1, and gene encoding the same

&lt;130&gt; 661562

5 &lt;150&gt; JP 10-290711

&lt;151&gt; 1998-10-13

&lt;160&gt; 13

&lt;210&gt; 1

10 &lt;211&gt; 2311

&lt;212&gt; DNA

&lt;213&gt; Rattus norvegicus

&lt;400&gt; 1

15 agagagagag agagagagag agagagagag agagagagaa atttgatttc cacagcatca 60  
 gctccttaag ggaaggtgag attcctaaga gatcagtaga gagcaccagg gagctcgctg 120  
 ctgtgttgct atggtgatga tggcaatggt aatgacagtg gcaccagatt tccctgttcc 180  
 tgggaagccc ctccggctcc cgcgggtggg cggcggcggc gatcgggtgc gcaaatccgc 240  
 gctcgcaccc gggcctgcgg ggcaggggcg cggcgtctga tttccttccc tgcctctgca 300  
 gccctgtgc gcatgctcgg cctacggcgc ccagccttt gattgatcgg tcggcagcgg 360  
 20 ctgcgaccct gggcggcaga cggcgggga tggggagccc ggcgctgga gcggcgcagt 420  
 gatcagcggg ggcggccggt gagtaccggt gagtaccgeg gc atg ggg ctc cgc 474

Met Gly Leu Arg

25 aag aag aac gcc agg aac ccc ccg gtg ctg agc cac gaa ttc atg gtg 522  
 Lys Lys Asn Ala Arg Asn Pro Pro Val Leu Ser His Glu Phe Met Val  
 5 10 15 20  
 cag aac cac gcg gat atg gtc tcc tgc gtg ggc atg ttc ttc gtg ctg 570  
 Gln Asn His Ala Asp Met Val Ser Cys Val Gly Met Phe Phe Val Leu

25

30

35

09807470.050401

gga ctt atg ttc gag ggc acg gcc gag atg tcg atc gtg ttc ctc acc 618  
 Gly Leu Met Phe Glu Gly Thr Ala Glu Met Ser Ile Val Phe Leu Thr  
                     40                    45                    50  
 ctg cag cat gga gtc gtt gtc cca gcg gaa ggg cta ccc tcg ggg tcc 666  
 5 Leu Gln His Gly Val Val Val Pro Ala Glu Gly Leu Pro Ser Gly Ser  
                     55                    60                    65  
 agg acc ctt tac cat tat ggg gtc aaa gat ctg gcc aca gtg ttc ttc 714  
 Arg Thr Leu Tyr His Tyr Gly Val Lys Asp Leu Ala Thr Val Phe Phe  
                     70                    75                    80  
 10 tac atg ctg gtg gcc atc atc att cac gcc acc att cag gag tac gtg 762  
 Tyr Met Leu Val Ala Ile Ile Ile His Ala Thr Ile Gln Glu Tyr Val  
                     85                    90                    95                    100  
 cta gat aag ctc agc cgg aga ctg cag ctc acc aaa ggc aaa caa aac 810  
 Leu Asp Lys Leu Ser Arg Arg Leu Gln Leu Thr Lys Gly Lys Gln Asn  
 15                                      105                    110                    115  
 aaa ttg aat gag gcc ggg cag ctg agt gtg ttc tac ata gtg tct ggt 858  
 Lys Leu Asn Glu Ala Gly Gln Leu Ser Val Phe Tyr Ile Val Ser Gly  
                     120                    125                    130  
 atc tgg ggt atg atc att ctg gcc tct gag aac tgc ctg tca gac ccc 906  
 20 Ile Trp Gly Met Ile Ile Leu Ala Ser Glu Asn Cys Leu Ser Asp Pro  
                     135                    140                    145  
 act cta ttg tgg aag tct cag ccc cac aac atg atg aca ttt cag atg 954  
 Thr Leu Leu Trp Lys Ser Gln Pro His Asn Met Met Thr Phe Gln Met  
                     150                    155                    160  
 25 aaa ttt ttc tac atc tca cag ttg gct tac tgg ttt cat agt ttc ccg 1002  
 Lys Phe Phe Tyr Ile Ser Gln Leu Ala Tyr Trp Phe His Ser Phe Pro  
                     165                    170                    175                    180  
 gag ctc tac ttc cag aaa gtc agg aaa caa gat atc ccg ggt caa ctc 1050  
 Glu Leu Tyr Phe Gln Lys Val Arg Lys Gln Asp Ile Pro Gly Gln Leu

0000470.0360  
 104050" 0440360



		185		190		195	
	atc tac att ggc ctc cac ctc ttc cac att gga ggg gcc tat ctc ttg						1098
	Ile Tyr Ile Gly Leu His Leu Phe His Ile Gly Gly Ala Tyr Leu Leu						
	200		205		210		
5	tac ttg aac cac ctg ggc ctg ctg ctt ctg atg ctg cac tat gct gtc						1146
	Tyr Leu Asn His Leu Gly Leu Leu Leu Met Leu His Tyr Ala Val						
	215		220		225		
	gag ctc ctc tcc agc gtg tgc agc ctg ctt tac ttt ggg gat gag cgg						1194
	Glu Leu Leu Ser Ser Val Cys Ser Leu Leu Tyr Phe Gly Asp Glu Arg						
10	230		235		240		
	tac cag aaa ggg ttg tct ttg tgg cct atc gtg ttt ata tcc ggg aga						1242
	Tyr Gln Lys Gly Leu Ser Leu Trp Pro Ile Val Phe Ile Ser Gly Arg						
	245		250		255		260
	ctc gtg aca ctg att gtc tca gtg gtt aca gta ggg ctt cac ttg gcc						1290
15	Leu Val Thr Leu Ile Val Ser Val Val Thr Val Gly Leu His Leu Ala						
	265		270		275		
	ggg aca aat cgg aat gga aat gct ctc tct ggt aat gtc aat gtg ttg						1338
	Gly Thr Asn Arg Asn Gly Asn Ala Leu Ser Gly Asn Val Asn Val Leu						
	280		285		290		
20	gca gct aaa atc gct gtt ctg tcc tcg agt tgc agt atc cag gtg tac						1386
	Ala Ala Lys Ile Ala Val Leu Ser Ser Ser Cys Ser Ile Gln Val Tyr						
	295		300		305		
	ata aca tgg acc ttg acg acc gtc tgg ctt cag aga tgg tta gaa gat						1434
	Ile Thr Trp Thr Leu Thr Thr Val Trp Leu Gln Arg Trp Leu Glu Asp						
25	310		315		320		
	aat ctt cat gtc tgt ggg agg aag aga egg tcc agg teg aga aaa						1482
	Ala Asn Leu His Val Cys Gly Arg Lys Arg Arg Ser Arg Ser Arg Lys						
	325		330		335		340
	ggc aca gaa aat gga gtg gag aat cca aat aga ata gat tct cca cca						1530

Gly Thr Glu Asn Gly Val Glu Asn Pro Asn Arg Ile Asp Ser Pro Pro

345

350

355

aag aag aaa gag aaa gct cct tagcagttgc aagcgaattg attcttacct 1581

Lys Lys Lys Glu Lys Ala Pro

5

360

ccaaggggaat ccaacttcttc ttatgtggtg tctctgtgct agagattttc tgttcttcag 1641

aacgggtcgt gctttttgaa tattgctaata gtattgtcta atgtgttttt aaggttttgc 1701

agacgtatga gtgggggatg ggggttaaga ctaaaccact cagcctctaa atacagtcag 1761

aatagttaac ggaccaacat cttatttagt taggttctta cctcaacgat tttccaaacg 1821

10

ttttgtggtg atgactgcag aattgtgtac ataaataata gtttctctgct tccaatgttc 1881

tttatcgaat taacaagtct gctagcaaag tggtttggtt tctcaatgtt ctctgcagg 1941

ataaagtgga aaatctgata aaggttaaac tcaaatcagt attatgtaac cgttgggatt 2001

tttttaaagt gttttaaatt tacaatggaa agcatttgct aaaccaccaa aaatatgtgt 2061

ttaattttat gagtagtaat tgttagtgtc tacgccccca ttaaagcatc aaaatatgaa 2121

15

tagatgacat gtgtggtgat attgacattt agcgaatcaa gataccttta ataaatatgg 2181

tgggttacta aagaagtaaa cgacttcttc ctgtttattt taaacacttg tacaggaaaa 2241

ctcgcaaaat taaatattac tgaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2301

aaaaaaaaa 2311

20

<210> 2

<211> 363

<212> PRT

<213> Rattus norvegicus

<400> 2

25

Met Gly Leu Arg Lys Lys Asn Ala Arg Asn Pro Pro Val Leu Ser His

5

10

15

Glu Phe Met Val Gln Asn His Ala Asp Met Val Ser Cys Val Gly Met

20

25

30

Phe Phe Val Leu Gly Leu Met Phe Glu Gly Thr Ala Glu Met Ser Ile

09807470-050401

35 40 45  
 Val Phe Leu Thr Leu Gln His Gly Val Val Val Pro Ala Glu Gly Leu  
 50 55 60  
 Pro Ser Gly Ser Arg Thr Leu Tyr His Tyr Gly Val Lys Asp Leu Ala  
 5 65 70 75 80  
 Thr Val Phe Phe Tyr Met Leu Val Ala Ile Ile Ile His Ala Thr Ile  
 85 90 95  
 Gln Glu Tyr Val Leu Asp Lys Leu Ser Arg Arg Leu Gln Leu Thr Lys  
 100 105 110  
 10 Gly Lys Gln Asn Lys Leu Asn Glu Ala Gly Gln Leu Ser Val Phe Tyr  
 115 120 125  
 Ile Val Ser Gly Ile Trp Gly Met Ile Ile Leu Ala Ser Glu Asn Cys  
 130 135 140  
 Leu Ser Asp Pro Thr Leu Leu Trp Lys Ser Gln Pro His Asn Met Met  
 15 145 150 155 160  
 Thr Phe Gln Met Lys Phe Phe Tyr Ile Ser Gln Leu Ala Tyr Trp Phe  
 165 170 175  
 His Ser Phe Pro Glu Leu Tyr Phe Gln Lys Val Arg Lys Gln Asp Ile  
 180 185 190  
 20 Pro Gly Gln Leu Ile Tyr Ile Gly Leu His Leu Phe His Ile Gly Gly  
 195 200 205  
 Ala Tyr Leu Leu Tyr Leu Asn His Leu Gly Leu Leu Leu Leu Met Leu  
 210 215 220  
 His Tyr Ala Val Glu Leu Leu Ser Ser Val Cys Ser Leu Leu Tyr Phe  
 25 225 230 235 240  
 Gly Asp Glu Arg Tyr Gln Lys Gly Leu Ser Leu Trp Pro Ile Val Phe  
 245 250 255  
 Ile Ser Gly Arg Leu Val Thr Leu Ile Val Ser Val Val Thr Val Gly  
 260 265 270

09807470-03040  
T04050-02420860

Leu His Leu Ala Gly Thr Asn Arg Asn Gly Asn Ala Leu Ser Gly Asn

275

280

285

Val Asn Val Leu Ala Ala Lys Ile Ala Val Leu Ser Ser Ser Cys Ser

290

295

300

5 Ile Gln Val Tyr Ile Thr Trp Thr Leu Thr Thr Val Trp Leu Gln Arg

305

310

315

320

Trp Leu Glu Asp Ala Asn Leu His Val Cys Gly Arg Lys Arg Arg Ser

325

330

335

Arg Ser Arg Lys Gly Thr Glu Asn Gly Val Glu Asn Pro Asn Arg Ile

10

340

345

350

Asp Ser Pro Pro Lys Lys Lys Glu Lys Ala Pro

355

360

<210> 3

15

<211> 2288

<212> DNA

<213> Homo sapiens

<400> 3

tatagggcac gcgtggtcga cgccccgggc tggactggg attttgctgt tattattatg 60

20

ctattgttgt tataattaat gatctgaaga ataaccagag ctctataggt ttatcatgat 120

tactaatgaa gatgccacta aaaaaaagaa ttcaggagca tcttggcggg ggcagcgagt 180

ttgaagatgc gacgatcaac gttgaagatc accgctcgca accccggggc tggcgccggg 240

taggggcgcg gcgctcgatt tccttccctg cctccgccgt cccctgggtg cgcattctca 300

gctcagctcg gccctgcct ttgatttatt tttttctgg gcggccgctg cgaccgggga 360

25

ctgacttcgg gatgggaagt ggagcccccg gagctgctac cgtggcgcg cgctgtgag 420

gagcagccag ggggaggcag ctgcgctcg ccggtgagta tccgggaagc gccacc 476

atg ggg ctc cgt aag aag agc acc aag aac ccc ccc gtt ctc agc cag 524

Met Gly Leu Arg Lys Lys Ser Thr Lys Asn Pro Pro Val Leu Ser Gln

5

10

15

FOH050 0240860

25

	165	170	175	
	ttt cat gct ttt cct gaa ctc tac ttc cag aaa acc aaa aaa caa gac			1052
	Phe His Ala Phe Pro Glu Leu Tyr Phe Gln Lys Thr Lys Lys Gln Asp			
	180	185	190	
5	atc cct cgt caa ctt gtc tac att ggt ctt cac ctc ttc cac att act			1100
	Ile Pro Arg Gln Leu Val Tyr Ile Gly Leu His Leu Phe His Ile Thr			
	195	200	205	
	gga gct tat ctc ttg tac ttg aat cat ttg gga ctt ctt ctt ttg gta			1148
	Gly Ala Tyr Leu Leu Tyr Leu Asn His Leu Gly Leu Leu Leu Leu Val			
10	210	215	220	
	ctg cat tat ttt gtt gaa tta ctt tcc cac atg tgc ggc ctg ttt tac			1196
	Leu His Tyr Phe Val Glu Leu Leu Ser His Met Cys Gly Leu Phe Tyr			
	225	230	235	240
	ttt agt gat gaa aag tac cag aaa ggc ata tct ctg tgg gcc att gtg			1244
15	Phe Ser Asp Glu Lys Tyr Gln Lys Gly Ile Ser Leu Trp Ala Ile Val			
	245	250	255	
	ttt atc ttg ggt aga ctt gtg act tta att gtt tcc gta ctc act gtt			1292
	Phe Ile Leu Gly Arg Leu Val Thr Leu Ile Val Ser Val Leu Thr Val			
	260	265	270	
20	ggg ttt cac ctg gct gga tcg cag aat cgg aat cct gat gcc ctt act			1340
	Gly Phe His Leu Ala Gly Ser Gln Asn Arg Asn Pro Asp Ala Leu Thr			
	275	280	285	
	gga aat gta aat gtg ttg gca gct aaa att gct gtt ctg tcg tcc agt			1388
	Gly Asn Val Asn Val Leu Ala Ala Lys Ile Ala Val Leu Ser Ser Ser			
25	290	295	300	
	tgc acg atc caa gcc tac gta aca tgg aac tta att act ctc tgg ctt			1436
	Cys Thr Ile Gln Ala Tyr Val Thr Trp Asn Leu Ile Thr Leu Trp Leu			
	305	310	315	320
	cag agg tgg gta gaa gat tct aat att cag gcc tca tgt atg aaa aag			1484

09807470-050401

9/25

Gln Arg Trp Val Glu Asp Ser Asn Ile Gln Ala Ser Cys Met Lys Lys

325

330

335

aaa cgg tcg aga tct tct aaa aaa aga aca gaa aac gga gtg gga gtg 1532

Lys Arg Ser Arg Ser Ser Lys Lys Arg Thr Glu Asn Gly Val Gly Val

5

340

345

350

gaa act tca aat aga gta gac tgt ccg cca aag agg aaa gag aaa tct 1580

Glu Thr Ser Asn Arg Val Asp Cys Pro Pro Lys Arg Lys Glu Lys Ser

355

360

365

tca taatctttgc aagcgcttg attaattgtct gcaaaggaat ctgctctttg 1633

Ser

10

aggtttcttt ctgcactaga gatttttctg tttttgaaaa tagttcgtgc tcttcggttt 1693

ttgttattga actgtttcat gtatttttta aagacatttg aggggaggag gattattatg 1753

aatgggaaaa aaagattttg gttgagacta aattactcat cgtcaaaata atgtcaaaat 1813

15

agttttgggg atcaccacta tattttgttt tgatttttaa ctttcaaca ttttcctaat 1873

gatttgcaga gataactgca caattttgca tatcaatgat actggttctt actcccacca 1933

gtgtttcata atactaacia gatggtctct cctagcaaga ttatgtgttt aatgcttgct 1993

ttggggtaaa ataaaagtac gaaaaagggtg gaagtcaaat cagtattctg taattgtag 2053

aatttatttt ttaagaactt acaactcaga aaagattgct agactcacca aaataataaa 2113

20

tgttctttat ttacaggta gtgattatta gtgcttcac cccatttaaa aaaacacagt 2173

actaatgggt aacacatatg gaggtttgct gccatatata ttgcatcaaa atatcattaa 2233

ttaatataaa aatattaataa tcattcctgt ccattccact tgtaaatggg aattc 2288

<210> 4

25

<211> 369

<212> PRT

<213> Homo sapiens

<400> 4

Met Gly Leu Arg Lys Lys Ser Thr Lys Asn Pro Pro Val Leu Ser Gln

09807470.05404

10/25

5

10

15

Glu Phe Ile Leu Gln Asn His Ala Asp Ile Val Ser Cys Val Gly Met

20

25

30

Phe Phe Leu Leu Gly Leu Val Phe Glu Gly Thr Ala Glu Ala Ser Ile

5

35

40

45

Val Phe Leu Thr Leu Gln His Ser Val Ala Val Pro Ala Ala Glu Glu

50

55

60

Gln Ala Thr Gly Ser Lys Ser Leu Tyr Tyr Tyr Gly Val Lys Asp Leu

65

70

75

80

10

Ala Thr Val Phe Phe Tyr Met Leu Val Ala Ile Ile Ile His Ala Thr

85

90

95

Ile Gln Glu Tyr Val Leu Asp Lys Ile Asn Lys Arg Met Gln Phe Thr

100

105

110

Lys Ala Lys Gln Asn Lys Phe Asn Glu Ser Gly Gln Phe Ser Val Phe

15

115

120

125

Tyr Phe Phe Ser Cys Ile Trp Gly Thr Phe Ile Leu Ile Ser Glu Asn

130

135

140

Cys Leu Ser Asp Pro Thr Leu Ile Trp Lys Ala Arg Pro His Ser Met

145

150

155

160

20

Met Thr Phe Gln Met Lys Phe Phe Tyr Ile Ser Gln Leu Ala Tyr Trp

165

170

175

Phe His Ala Phe Pro Glu Leu Tyr Phe Gln Lys Thr Lys Lys Gln Asp

180

185

190

Ile Pro Arg Gln Leu Val Tyr Ile Gly Leu His Leu Phe His Ile Thr

25

195

200

205

Gly Ala Tyr Leu Leu Tyr Leu Asn His Leu Gly Leu Leu Leu Leu Val

210

215

220

Leu His Tyr Phe Val Glu Leu Leu Ser His Met Cys Gly Leu Phe Tyr

225

230

235

240

T04050" 02420360



11/25

Phe Ser Asp Glu Lys Tyr Gln Lys Gly Ile Ser Leu Trp Ala Ile Val

245

250

255

Phe Ile Leu Gly Arg Leu Val Thr Leu Ile Val Ser Val Leu Thr Val

260

265

270

5 Gly Phe His Leu Ala Gly Ser Gln Asn Arg Asn Pro Asp Ala Leu Thr

275

280

285

Gly Asn Val Asn Val Leu Ala Ala Lys Ile Ala Val Leu Ser Ser Ser

290

295

300

Cys Thr Ile Gln Ala Tyr Val Thr Trp Asn Leu Ile Thr Leu Trp Leu

10 305

310

315

320

Gln Arg Trp Val Glu Asp Ser Asn Ile Gln Ala Ser Cys Met Lys Lys

325

330

335

Lys Arg Ser Arg Ser Ser Lys Lys Arg Thr Glu Asn Gly Val Gly Val

340

345

350

15 Glu Thr Ser Asn Arg Val Asp Cys Pro Pro Lys Arg Lys Glu Lys Ser

355

360

365

Ser

20 <210> 5

<211> 1267

<212> DNA

<213> Homo sapiens

<400> 5

25 cagcgagcgg ctgcagcggg gccgtgacca gcagccagcg ggaggcggcg gcgagtcggt 60

gagcagctgg gaagagcaga accggggcgg agcacctgca ggcgcgggcg gcggccccac 120

c atg gcg att cgc aag aaa agc acc aag agc ccc cca gtg ctg agc 166

Met Ala Ile Arg Lys Lys Ser Thr Lys Ser Pro Pro Val Leu Ser

5

10

15

09807470.050401

12/25

	cac gaa ttc gtc ctg cag aat cac ggc gac atc gtc tcc tgt gtg ggc	214
	His Glu Phe Val Leu Gln Asn His Ala Asp Ile Val Ser Cys Val Ala	
	20 25 30	
	atg gtc ttc ctg ctg ggg ctc atg ttt gag ata acg gca aaa gct tct	262
5	Met Val Phe Leu Leu Gly Leu Met Phe Glu Ile Thr Ala Lys Ala Ser	
	35 40 45	
	atc att ttt gtt act ctt cag tac aat gtc acc ctc cca gca aca gaa	310
	Ile Ile Phe Val Thr Leu Gln Tyr Asn Val Thr Leu Pro Ala Thr Glu	
	50 55 60	
10	gaa caa gct act gaa tca gtg tcc ctt tat tac tat ggc atc aaa gat	358
	Glu Gln Ala Thr Glu Ser Val Ser Leu Tyr Tyr Tyr Gly Ile Lys Asp	
	65 70 75	
	ttg gct act gtt ttc ttc tac atg cta gtg ggc ata att att cat gcc	406
	Leu Ala Thr Val Phe Phe Tyr Met Leu Val Ala Ile Ile Ile His Ala	
15	80 85 90 95	
	gta att caa gag tat atg ttg gat aaa att aac agg cga atg cac ttc	454
	Val Ile Gln Glu Tyr Met Leu Asp Lys Ile Asn Arg Arg Met His Phe	
	100 105 110	
	tcc aaa aca aaa cac agc aag ttt aat gaa tct ggt cag ctt agt ggc	502
20	Ser Lys Thr Lys His Ser Lys Phe Asn Glu Ser Gly Gln Leu Ser Ala	
	115 120 125	
	ttc tac ctt ttt gcc tgt gtt tgg ggc aca ttc att ctc atc tct gaa	550
	Phe Tyr Leu Phe Ala Cys Val Trp Gly Thr Phe Ile Leu Ile Ser Glu	
	130 135 140	
25	aac tac atc tca gac cca act atc tta tgg agg gct tat ccc cat aac	598
	Asn Tyr Ile Ser Asp Pro Thr Ile Leu Trp Arg Ala Tyr Pro His Asn	
	145 150 155	
	ctg atg aca ttt caa atg aag ttt ttc tac ata tca cag ctg gct tac	646
	Leu Met Thr Phe Gln Met Lys Phe Phe Tyr Ile Ser Gln Leu Ala Tyr	

0980470-050401

160                      165                      170                      175  
 tgg ctt cat gct ttt cct gaa ctc tac ttc cag aaa acc aaa aaa gaa      694  
 Trp Leu His Ala Phe Pro Glu Leu Tyr Phe Gln Lys Thr Lys Lys Glu  
                          180                      185                      190  
 5      gat att cct cgt cag ctt gtc tac att ggt ctt tac ctc ttc cac att      742  
 Asp Ile Pro Arg Gln Leu Val Tyr Ile Gly Leu Tyr Leu Phe His Ile  
                          195                      200                      205  
 gct gga gct tac ctt ttg aac ttg aat cat cta gga ctt gtt ctt ctg      790  
 Ala Gly Ala Tyr Leu Leu Asn Leu Asn His Leu Gly Leu Val Leu Leu  
 10                      210                      215                      220  
 gtg cta cat tat ttt gtt gaa ttt ctt ttc cac att tcc cgc ctg ttt      838  
 Val Leu His Tyr Phe Val Glu Phe Leu Phe His Ile Ser Arg Leu Phe  
                          225                      230                      235  
 tat ttt agc aat gaa aag tat cag aaa gga ttt tct ctg tgg gca gtt      886  
 15      Tyr Phe Ser Asn Glu Lys Tyr Gln Lys Gly Phe Ser Leu Trp Ala Val  
 240                      245                      250                      255  
 ctt ttt gtt ttg gga aga ctt ctg act tta att ctt tca gta ctg act      934  
 Leu Phe Val Leu Gly Arg Leu Leu Thr Leu Ile Leu Ser Val Leu Thr  
                          260                      265                      270  
 20      gtt ggt ttt ggc ctt gca aga gca gaa aat cag aaa ctg gat ttc agt      982  
 Val Gly Phe Gly Leu Ala Arg Ala Glu Asn Gln Lys Leu Asp Phe Ser  
                          275                      280                      285  
 act gga aac ttc aat gtg tta gct gtt aga atc gct gtt ctg gca tcc      1030  
 Thr Gly Asn Phe Asn Val Leu Ala Val Arg Ile Ala Val Leu Ala Ser  
 25                      290                      295                      300  
 att tgc gtt act cag gca ttt atg atg tgg aag ttc att aat ttt cag      1078  
 Ile Cys Val Thr Gln Ala Phe Met Met Trp Lys Phe Ile Asn Phe Gln  
                          305                      310                      315  
 ctt cga agg tgg agg gaa cat tct gct ttt cag gca cca gct gtg aag      1126

09807470-050401

Leu Arg Arg Trp Arg Glu His Ser Ala Phe Gln Ala Pro Ala Val Lys

320 325 330 335

aag aaa cca aca gta act aaa ggc aga tct tct aaa aaa gga aca gaa 1174

Lys Lys Pro Thr Val Thr Lys Gly Arg Ser Ser Lys Lys Gly Thr Glu

5 340 345 350

aat ggt gtg aat gga aca tta act tca aat gta gca gac tct ccc cgg 1222

Asn Gly Val Asn Gly Thr Leu Thr Ser Asn Val Ala Asp Ser Pro Arg

355 360 365

aat aaa aaa gag aaa tct tca taatgaatta taaactaatt gatt 1267

10 Asn Lys Lys Glu Lys Ser Ser

370

<210> 6

<211> 374

15 <212> PRT

<213> Homo sapiens

<400> 6

Met Ala Ile Arg Lys Lys Ser Thr Lys Ser Pro Pro Val Leu Ser His

5 10 15

20 Glu Phe Val Leu Gln Asn His Ala Asp Ile Val Ser Cys Val Ala Met

20 25 30

Val Phe Leu Leu Gly Leu Met Phe Glu Ile Thr Ala Lys Ala Ser Ile

35 40 45

Ile Phe Val Thr Leu Gln Tyr Asn Val Thr Leu Pro Ala Thr Glu Glu

25 50 55 60

Gln Ala Thr Glu Ser Val Ser Leu Tyr Tyr Tyr Gly Ile Lys Asp Leu

65 70 75 80

Ala Thr Val Phe Phe Tyr Met Leu Val Ala Ile Ile Ile His Ala Val

85 90 95

Ile Gln Glu Tyr Met Leu Asp Lys Ile Asn Arg Arg Met His Phe Ser

100

105

110

Lys Thr Lys His Ser Lys Phe Asn Glu Ser Gly Gln Leu Ser Ala Phe

115

120

125

5 Tyr Leu Phe Ala Cys Val Trp Gly Thr Phe Ile Leu Ile Ser Glu Asn

130

135

140

Tyr Ile Ser Asp Pro Thr Ile Leu Trp Arg Ala Tyr Pro His Asn Leu

145

150

155

160

Met Thr Phe Gln Met Lys Phe Phe Tyr Ile Ser Gln Leu Ala Tyr Trp

10

165

170

175

Leu His Ala Phe Pro Glu Leu Tyr Phe Gln Lys Thr Lys Lys Glu Asp

180

185

190

Ile Pro Arg Gln Leu Val Tyr Ile Gly Leu Tyr Leu Phe His Ile Ala

195

200

205

15 Gly Ala Tyr Leu Leu Asn Leu Asn His Leu Gly Leu Val Leu Leu Val

210

215

220

Leu His Tyr Phe Val Glu Phe Leu Phe His Ile Ser Arg Leu Phe Tyr

225

230

235

240

Phe Ser Asn Glu Lys Tyr Gln Lys Gly Phe Ser Leu Trp Ala Val Leu

20

245

250

255

Phe Val Leu Gly Arg Leu Leu Thr Leu Ile Leu Ser Val Leu Thr Val

260

265

270

Gly Phe Gly Leu Ala Arg Ala Glu Asn Gln Lys Leu Asp Phe Ser Thr

275

280

285

25 Gly Asn Phe Asn Val Leu Ala Val Arg Ile Ala Val Leu Ala Ser Ile

290

295

300

Cys Val Thr Gln Ala Phe Met Met Trp Lys Phe Ile Asn Phe Gln Leu

305

310

315

320

Arg Arg Trp Arg Glu His Ser Ala Phe Gln Ala Pro Ala Val Lys Lys

09807470-050401

325

330

335

Lys Pro Thr Val Thr Lys Gly Arg Ser Ser Lys Lys Gly Thr Glu Asn

340

345

350

Gly Val Asn Gly Thr Leu Thr Ser Asn Val Ala Asp Ser Pro Arg Asn

5

355

360

365

Lys Lys Glu Lys Ser Ser

370

&lt;210&gt; 7

10

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 7

cacctggctg gatcgagaa tcgg

24

15

&lt;210&gt; 8

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

20

&lt;400&gt; 8

ctctttctc tttggcggac agtc

24

&lt;210&gt; 9

&lt;211&gt; 24

25

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 9

ggccttgcaa gagcagaaaa tcag

24

T04050" 02420860

&lt;210&gt; 10

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

5 &lt;400&gt; 10

tttattccgg ggagagtctg ctac

24

&lt;210&gt; 11

&lt;211&gt; 49

10 &lt;212&gt; DNA

&lt;213&gt; Rattus norvegicus

&lt;400&gt; 11

attttctgtg ccttttctcg acctggaccg tctcttctc ccacagaca

49

15 &lt;210&gt; 12

&lt;211&gt; 6974

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 12

20 cgggtgctgga gaagtttgcg ctgcggttcg tgagcgcagg gtgcggggccc cgccggccgc 60

tgccgcgccg ctgcc atg gct ttc cgc agg agg acg aaa agt tac ccg ctc 111

Met Ala Phe Arg Arg Arg Thr Lys Ser Tyr Pro Leu

5

10

ttc agc cag gag ttc gtc atc cac aac cat gcg gac atc ggc ttc tgc 159

25 Phe Ser Gln Glu Phe Val Ile His Asn His Ala Asp Ile Gly Phe Cys

15

20

25

ctg gtg ctc tgc gtc ctc atc ggg ctt atg ttc gag gtc aca gcc aag 207

Leu Val Leu Cys Val Leu Ile Gly Leu Met Phe Glu Val Thr Ala Lys

30

35

40

T04050-02420860

	act gcc ttt cta ttt att tta cct cag tat aac att agc gtg cct aca	255
	Thr Ala Phe Leu Phe Ile Leu Pro Gln Tyr Asn Ile Ser Val Pro Thr	
	45 50 55 60	
	gca gac agt gag acc. gtg cac tac cac tat ggc cct aag gac ctg gtc	303
5	Ala Asp Ser Glu Thr Val His Tyr His Tyr Gly Pro Lys Asp Leu Val	
	65 70 75	
	aca atc ttg ttc tac atc ttc atc acc atc atc ttg cat gct gtg gtt	351
	Thr Ile Leu Phe Tyr Ile Phe Ile Thr Ile Ile Leu His Ala Val Val	
	80 85 90	
10	cag gag tac att tta gat aaa atc agc aaa cgg ctt cat ctc tcc aaa	399
	Gln Glu Tyr Ile Leu Asp Lys Ile Ser Lys Arg Leu His Leu Ser Lys	
	95 100 105	
	gtc aaa cac agc aag ttc aat gaa tct gga cag ctg gtc gtc ttt cat	447
	Val Lys His Ser Lys Phe Asn Glu Ser Gly Gln Leu Val Val Phe His	
15	110 115 120	
	ttc acc tcg gtg att tgg tgc ttc tac gtg gtg gtg acg gaa gga tac	495
	Phe Thr Ser Val Ile Trp Cys Phe Tyr Val Val Val Thr Glu Gly Tyr	
	125 130 135 140	
	tta aca aac cca aga agc ctc tgg gaa gac tac ccg cat gtg cac ctc	543
20	Leu Thr Asn Pro Arg Ser Leu Trp Glu Asp Tyr Pro His Val His Leu	
	145 150 155	
	ccc ttc cag gtg aag ttt ttc tac cta tgc cag ctg gcc tac tgg ctg	591
	Pro Phe Gln Val Lys Phe Phe Tyr Leu Cys Gln Leu Ala Tyr Trp Leu	
	160 165 170	
25	cac gca ctt cct gag cta tac ttc cag aag gta cgg aag gag gaa att	639
	His Ala Leu Pro Glu Leu Tyr Phe Gln Lys Val Arg Lys Glu Glu Ile	
	175 180 185	
	ccc cgc cag ctc cag tat att tgc ctg tac ctg gtg cat ata gct gga	687
	Pro Arg Gln Leu Gln Tyr Ile Cys Leu Tyr Leu Val His Ile Ala Gly	



25

His Glu Asn Gly Val Val Lys Ala Glu Asn Gly Thr Ser Pro Arg Thr

350

355

360

aag aaa ctc aag tct ccc taaggccaaa gtgctaagaa caggaatcct 1215

Lys Lys Leu Lys Ser Pro

5

365

370

cttggtgggg gccgagcagg gggcaaggag cccaggcccc ctccctgcct cctccttcct 1275

gcctgtgatg ctccgtctca aacagccgaa acctgtcttg caatgggggg agggggcggt 1335

tcgctttcct tcttcttggc ttctcttat tctccacaa accattctca ataaagccaa 1395

aaatctttct ctttctcccc ctgagccac ctctgtcct cactcctgtc ctgtgctggc 1455

10

ttttctggaa cgccaggcgc ccattgctgg cactttctg ctgctctgt ttcttgccct 1515

atggtctgtg cttttccttt ttacttcta tttcacctt atcttgcaat tttctgtct 1575

gatttttaca atgggagggg agctaagatt gcagtcctgt ccttcgggtc cccagggcct 1635

gccggtcaga agcctggggc tggtaggcc ttggtgggtc tcatgtggat gggcaagaag 1695

agagcggoca tctcgatca taatctcctt ggtgctgatt aactgacgag atatatgatt 1755

15

ccagttctgc atgtaccatc ttgaggcaca gcagccactg ctgcttgtaa atgccaaggc 1815

atttggtttt gggacgtgac aactcaatcc agaaggatgg tgtgaactcg gttgggtccc 1875

gtgactcgag ctctaccag tggctggccg cggattggaa gccagcctgc tgcgctctg 1935

tggggaggac atgtcttccc actgcttaga gcgagagcag agcaaactgc gcagcaggca 1995

cctccagaaa ggtaatggtg gcagaaccca cagtggagtc gacctaggcc tttctccagc 2055

20

agtcccagtc gccattgctt tttcagccat tcacaagcat tcaaaaccaa accaaacagc 2115

agttcatata cctgcctgag ataggctggt cctcacctcc agagccagcc agccccgtca 2175

ggggccaaac ttactacctt gacttcatct ctagctgcag aaacactaag tctcaagggc 2235

ttcagcccca tgctgggtccc ttggtgttca gggagggtca cttggaccgc tgttcatctg 2295

gccgcccctg ttgagtgttc tttggaattg tcgttttttg agcacaacta cagcatttta 2455

25

gactgcatga aaccatgact gactgagagt cactctctgg gtagatgata ggccgctttc 2415

tggccccttc cctcacagat tctttccct cccctccacc tgaagagaag gcctccaagt 2475

ccttttggtg ctttgtgagg acttttagaa ggggcgttca gctttaaaaa gccggtccta 2535

attacggccg gacgcagtag cttacgcctg ttatccagc actttgggag gtcgaggtgg 2595

gcagatcacc tgaggttagg agttcaagac cagcctggcc aacatggtga aaccccatct 2655

09007470-050404

	ctactaaaaa tacaaaaaat taggtgtagt ggcaggcacc tgtaatccca gctactcggg	2715
	aggctgaggc aggagaatcg cttgaaccta gaaggtaggag gttgcagtga gcggagattg	2775
	taccatggca ctccagcctg gacaacaaga gcgaaattct gtctaaaaaa acaaaagtec	2835
	caattaagaa cctccgaact ctgttttgag gcaaagggga gtagttcttg gtaggtgcag	2895
5	gaatagtagt gtcatttgga atactggtea tctttctgac atcacagtag aaaccaaacc	2955
	ttggatttag attcaaaagg ggggaaatgg gtcttttcat caaggcaact ccccttctcc	3015
	aagtcactta catcatagat aaatttttagc ttcccagtaa ctgagggatt tgtttcctaa	3075
	cgccattgga ggccttcac cctctctacg ataaggttgc agaaatggga agagctaccc	3135
	gtggttgctt ttgattaccc ttaggaagtg agacagtgtt ttgaaaata tgtatttctc	3195
10	ccatttctcc ctctccttcc ctgacacttc tctgggctgc acagcagaaa cgttggtaaa	3255
	agggcagttt ggttcaaca cagcagacct gatatgggat cccttagcca ctttagtcaa	3315
	acagccctga cagagtctat aattgagttc agggccccca ccttgccctaa taactgcaaa	3375
	tgcattgttc agccagcagc ctctaagcc caccttcctc cccattaga gaacacccat	3435
	cctaggtgct ctccaggctg tgcattggc agggcttcac atgcaggagg cctctctcag	3495
15	gtgagtcag gttaaactgt tgagttgtgg cttcaacaga tatgtatggc atgctgggat	3555
	gtgccaggtg cctgcgttgt gccagttgct ggagaggtag tgtgagcaga gcagctgaaa	3615
	tcttgccatc aagcaaccct cattctcatg cctgtaggtt tccattgctc tgtcccagga	3675
	cacttgctg ccagagacgc cacaacttca tgtccctgtc tcttgcaagc tcccgtgct	3735
	gccagtactt catgccttgg atgttgtccc accagcccag tggctgggggt cagcttaggc	3795
20	tctgcttccc agtggacggg tgtgctaagg gtttatttta tgtaaaaaaa aaaaaaaaaac	3855
	aaaaaaaaac cctgagacca tgagtggggc tggcatcttg ccagcctggg cttcagggat	3915
	gtttgggggg ggtggttaga gggtagttgt agggctacttt gtcaccccc tccccctgcc	3975
	accctccctg gcacgtttat ttcacagcag agccaagtct gtggcaggtt gacacagact	4035
	gtgttgccag agctgaaata attccacttc atcctatgag cgtgttgggc tagcttgttc	4095
25	taattttggc cactttggct gttttcttca gttttatgca ttctctcctg ccccaaagt	4155
	ccaagccatt tgtgaaggct ctgccagaca cctccaagct tgagagctca gcaccatgca	4215
	ccaagagcag gagaaaagac gtaaacctac cccagcaact gtggcctctc gacagccctg	4275
	gctaactaac ttacatttgt ggggaagcca acagacacag caggaggaga gggaggtggc	4335
	gctggtggac caaggatctg tgetaccgc tcccctcctt ggaggtgcag tgatgatggg	4395

agttatTTTT accatccggg cgctgatagc tgcactatta ataaattgca tgtgttcctt 4455  
 ttgaaggtag gggatggttc tgggtgagag gggagcaggc tgagccggcg ggggatctgc 4515  
 tgtctccct tttgagtcag ttctaatecc atgtgtgtct gggccaccag accgaaatgg 4575  
 ttgctgagaa acttgtctgt tcatgtccca aggcataact tcccaacatt taagaaaccc 4635  
 5 caatagacac ctctgccctg gccacgttca cagatccttc tcttgaccgg aaacctggg 4695  
 accctaagaa cccctgaagc ttgggggtgg tgtgtgcttc tggggctctt tttgggacct 4755  
 cctttgtcag tacccttct tttttctaag cagctaataa gaggttgggt gaaagagtgc 4815  
 atctcctccc aggattccac aacaaaattc ttatcttcca tggatgcttt aattggaagt 4875  
 gggttgccga ccccttgtg cctagaaaag gcctttgctt gggtttcctt tgtatgcttc 4935  
 10 agccttccta gttggttttt ctaggcctgg tgtgagaggt agggaagtct gcacataact 4995  
 aattcttttg ctttaagggc tatggcacia gtgcaciaac ttcaattctt gatgttctaa 5055  
 gctctctcct ctaacagagg gagtgtgaa agcttttgag tcaagacaat ggagtgtctt 5115  
 tctccctca ctctgccttc cgagcttatg gttccttttc tcaggagagg attttcagga 5175  
 ttattggagg attaggtcat tgtcagatga ctggaaaacc taaataggat ctctctccag 5235  
 15 ctcaagggtg tccagtgag gaagacttta ccaacttctc actctacccc actactcaca 5295  
 tgagtgttag ctccacctg caaaggetga agaccagttc tccccagtga aagctgcctc 5355  
 attcttttat ggagttccct ggagtggcag agctataaag acgagcattg ggatttgcag 5415  
 tctccatgta gcctttcgtg cttggcaacc cctgtagact ttttgtccca agcagattgc 5475  
 gtgcgtgcgc ctgtgtgtga gaataagtgc ctactttgc tgtgtgggtt tcaacttgta 5535  
 20 ctccgtggcc agccccagt tgccagggtc cgacggcagc caaggacacc atacctcagt 5595  
 atagttatat ataaaatgga cacggattgt gacagtttca cccatttgt ttctaacccc 5655  
 gctgcccagg attaggtct gtggtgtgtt ctgttttgtt tttggttct ccttgtgtc 5715  
 agttctcttc tggcccagct ggggtgctgt ggaagtctgt gaggtggccc aaccacaagc 5775  
 atacctatta agagaagccc agagcttcca gccccactt cgaaaactct cctctggccc 5835  
 25 cacatagcaa actccttctc cgttattttc cccaccccca gattttttt aaaaggccca 5895  
 cttgccataa cctcttttgg tctattttgc ttccattca gcccaaagtt tatatgataa 5955  
 aggtgtttac tttacttcc cagtctccaa gtgctaacac ataaacacat acatgtctga 6015  
 ctgttgcaga actgttcgag ctccataatc agtgttacct tgttttagtc gcagcaaccc 6075  
 tctccctac ccttgcccg cccacgtttt tctactctt ccgggttgtg caataactct 6135

cccagccagt ggtcctttcc acagcctttc tgtcccttaa aacacctgca actgggggag 6195  
 aaatgggacc catgggaggg ggagtcacaa tcccttacac aagaaatagc cactttcctt 6255  
 ttgttgatcat tcttgatc ctgggtgggt ttctgtggca ctcttttaga acatgtagca 6315  
 tcatcttaga ggtctatctt taaaaaatgt gttgaagagg aaaaaacat tctcacgatg 6375  
 5 gggcttaagt cattgtccag gaataagatt ggcggtgtgc ccatgacatc accgtcactc 6435  
 tgcctaaaag cactctagag ctacttggtc acgtggagag gaaggatatt ttggaagca 6495  
 acagccgcag gtggagagcc ctgttcacct gatagggtct agctgtgaca gtaaataata 6555  
 taccgtgtt tcttgggta cagatttgag tgttcagtgt atgagactgt aaacctcatt 6615  
 tttcggttcc tctgttttaa aaaacatctg aaggatgaac taaggctgct ggtgccctga 6675  
 10 gcaactgata atgcaaagt ggacaaagt tctgttttct actctagcct gttcatatgg 6735  
 accaaatttc aacaaggaa tcaaggaaaa tttgtacctg cgtatttat gctttcatgt 6795  
 aaaaaagggt tggggggagg ggtgtctttt tgcttttggt gaactttttt tcaaatcat 6855  
 tttccactg tttctgtctg gttttaaacc aaattacagt tttgtatgga ttttttaaat 6915  
 gtacattttg gaacaaatga tcaaatattt tctgaaataa caataaagg cagaaaatt 6974

<210> 13

<211> 370

<212> PRT

<213> Homo sapiens

<400> 13

Met Ala Phe Arg Arg Arg Thr Lys Ser Tyr Pro Leu Phe Ser Gln Glu

5

10

15

Phe Val Ile His Asn His Ala Asp Ile Gly Phe Cys Leu Val Leu Cys

20

25

30

Val Leu Ile Gly Leu Met Phe Glu Val Thr Ala Lys Thr Ala Phe Leu

35

40

45

Phe Ile Leu Pro Gln Tyr Asn Ile Ser Val Pro Thr Ala Asp Ser Glu

50

55

60

Thr Val His Tyr His Tyr Gly Pro Lys Asp Leu Val Thr Ile Leu Phe

004050-02420860

	65	70	75	80
	Tyr Ile Phe Ile Thr Ile Ile Leu His Ala Val Val Gln Glu Tyr Ile			
	85	90	95	
	Leu Asp Lys Ile Ser Lys Arg Leu His Leu Ser Lys Val Lys His Ser			
5	100	105	110	
	Lys Phe Asn Glu Ser Gly Gln Leu Val Val Phe His Phe Thr Ser Val			
	115	120	125	
	Ile Trp Cys Phe Tyr Val Val Val Thr Glu Gly Tyr Leu Thr Asn Pro			
	130	135	140	
10	Arg Ser Leu Trp Glu Asp Tyr Pro His Val His Leu Pro Phe Gln Val			
	145	150	155	160
	Lys Phe Phe Tyr Leu Cys Gln Leu Ala Tyr Trp Leu His Ala Leu Pro			
	165	170	175	
	Glu Leu Tyr Phe Gln Lys Val Arg Lys Glu Glu Ile Pro Arg Gln Leu			
15	180	185	190	
	Gln Tyr Ile Cys Leu Tyr Leu Val His Ile Ala Gly Ala Tyr Leu Leu			
	195	200	205	
	Asn Leu Ser Arg Leu Gly Leu Ile Leu Leu Leu Leu Gln Tyr Ser Thr			
	210	215	220	
20	Glu Phe Leu Phe His Thr Ala Arg Leu Phe Tyr Phe Ala Asp Glu Asn			
	225	230	235	240
	Asn Glu Lys Leu Phe Ser Ala Trp Ala Ala Val Phe Gly Val Thr Arg			
	245	250	255	
	Leu Phe Ile Leu Thr Leu Ala Val Leu Ala Ile Gly Phe Gly Leu Ala			
25	260	265	270	
	Arg Met Glu Asn Gln Ala Phe Asp Pro Glu Lys Gly Asn Phe Asn Thr			
	275	280	285	
	Leu Phe Cys Arg Leu Cys Val Leu Leu Leu Val Cys Ala Ala Gln Ala			
	290	295	300	

T04050-0242050

Trp Leu Met Trp Arg Phe Ile His Ser Gln Leu Arg His Trp Arg Glu

305 310 315 320

Tyr Trp Asn Glu Gln Ser Ala Lys Arg Arg Val Pro Ala Thr Pro Arg

325 330 335

5 Leu Pro Ala Arg Leu Ile Lys Arg Glu Ser Gly Tyr His Glu Asn Gly

340 345 350

Val Val Lys Ala Glu Asn Gly Thr Ser Pro Arg Thr Lys Lys Leu Lys

355 360 365

Ser Pro

10 370

0000420000